

**CRANFIELD UNIVERSITY**

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**POSTHARVEST BIOCHEMICAL AND TEXTURAL  
CHARACTERISTICS OF *sh2* SWEETCORN COBS**

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CHARACTERISTICS OF *sh2* SWEETCORN COBS

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## ABSTRACT

The determination of storage conditions leading to optimum quality attributes and extended postharvest life of sweetcorn is essential. The increased consumption and consequently the need for greater consumer satisfaction have resulted in the introduction of supersweet sweetcorn. The present study, aimed to report on the effects of various postharvest factors on biochemical and texture-related characteristics of supersweet sweetcorn cultivars as current knowledge is still incomplete.

Validation and optimisation of commonly used methods for the analysis of target compounds in sweetcorn was also an objective of the current work. The methods developed for the analysis of the target analytes (*viz.* ferulic acid, individual carotenoids, non-structural carbohydrates and vitamin C) were considered suitable. However, the Folin-Ciocalteu assay and the ferric ion reducing power assay, as methods for the determination of total phenolic content and total antioxidant capacity of sweetcorn, respectively, were shown as not appropriate for sweetcorn analysis.

Documentation on firmness and biochemical compounds as affected by cooking and the interaction with various storage conditions is still limited. The current project was the first to extensively monitor quality changes in firmness, and other quality attributes under cooking conditions. Results revealed that increased cooking time resulted in greater ferulic acid content and a significant decline in firmness and quality-related target analytes such as L-ascorbic acid and carotenoids, yet no change in sugars was observed. The firmest kernels were reported to be those located in the central part of the cobs, which is also the preferred edible portion for consumers. Surprisingly, spatial sugar profiles indicated higher total sugar content in non-edible tissues (*viz.* core and shank), rather than kernels and the implications of this are discussed.

Predictably, higher storage temperature and the longer storage period resulted in lower quality, yet genotype, controlled atmosphere and origin of the cobs were significant sources of variation for sugar content and firmness of kernels. The presence of husks (*i.e.* non-removal) on sweetcorn cobs promoted the retention of sugar content and colour over storage. Recommendations for improved methods for the measurement

of target analytes leading to valid conclusions about optimum storage conditions are also included.

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combined storage temperature, format and cooking time.

## NOTATION

<	less than
>	greater than
%	per cent
=	equal to
A	alpha
AAO	Ascorbate acid oxidase
ACN	acetonitrile
ADPG	Adenosine 5'-diphosphate glucose phosphorylase
AMG	amyloglucosidase
ANOVA	Analysis of Variance
AU	arbitrari units
<i>B</i>	beta
Berks.	Berkshire
BHT	butylated hydroxytoluene
BOC	British Oxygen Company
<i>Btl</i>	brittle
°C	degree Celsius
Ca	calcium
CA.	California
CA	Controlled Atmosphere
<i>ca.</i>	approximately
CAC	cellular antioxidant activity
C*	Chroma
Cm	centimetre
CO <sub>2</sub>	Carbon dioxide
Corp.	Corporation
Cu	Copper

CV	Coefficient of variation
cv.	cultivar
DAD	diode array detector
d.f.	degrees of freedom
DHAA	dehydroascorbic acid
DW	dry weight
EH	Everlasting Heritage
<i>et al.</i>	and others
EU	European Union
ELSD	Evaporative light scattering detector
Exp.	Experiment
FAS	Foreign Agricultural Service
Fe	Iron
FeCl <sub>3</sub>	Iron(III) chloride
FRAP	ferrous reducing antioxidant power
FW	fresh weight
g.	gravity
G	gram
GAE	gallic acid equivalents
GC	Gas Chromatography
H	hour
H°	Hue angle
HCl	Hydrochloric acid
HMF	5-hydroxymethylfurfural
HPLC	High Performance Liquid Chromatography
K	potassium
KOH	potassium hydroxide
KPa	KiloPascal
L	litre
L*	Lightness
Lab	laboratory
Leics.	Leicester

LSD	least significant difference
Ltd	Private company limited by shares
M	Molarity
MA	Modified Atmosphere
MA.	Massachusetts
MAP	Modified atmosphere packaging
MeOH	methanol
Min	minute
Mg	micrograms
Mg	milligram
mL	millilitre
$\mu$ L	microlitre
Mm	micrometre
mM	millimolar
Mm	millimetre
Mo	molybdenum
Mol	mole
MT	metric tonnes
MTT	3-4,5-dimethylthiazolyl-2-2,5-diphenyltetrazolium bromide
N	Number
N	Newton
Na	Sodium
Ni	Nickel
Nm	nanometre
NSC	non-structural carbohydrate
NY	New York
O <sub>2</sub>	Oxygen
ORAC	oxygen radical absorbance capacity
pH	a measure of acidity or basicity of a solution
<i>P</i>	Probability
Pa	Pascal

PDS	phytoene desaturase
PMS	phenazynemethosulphate
POD	peroxidase
RID	refractive index detector
S.D.	standard deviation
S.E.	standard error
Sen.	Senegal
<i>sh2</i>	<i>Shrunk-en-2</i>
Staffs.	Stafford
T	temperature
TEA	triethylamine
TEAC	Trolox equivalent antioxidant capacity
TOSC	total oxyradical scavenging capacity
TP	total phenolics
TSS	Total Soluble Solids
UK	United Kingdom
US	United States
USA	United States of America
USDA	United States Department of Agriculture
UV	ultraviolet
<i>Var.</i>	variety
VCEAC	Vitamin C equivalent antioxidant capacity
Vis	visible
<i>viz.</i>	namely
Vs.	versus
v/v	volume per volume
W.	west
WS	Window Stripped
WSP	Water Soluble Polysaccharides
w/v	weight per volume
<i>Wx</i>	waxy
Zn	Zinc



# 1. CHAPTER ONE

## Introduction

### 1.1 Project background

Sweetcorn (*Zea mays L.*) is an important crop that initially was a very popular vegetable in US and Asia, but recently also in Europe. During the last two decades the consumption and production of sweetcorn has been increased significantly. Postharvest treatments directly affect the quality presented to consumers. As a consequence appropriate storage conditions are paramount to ensure the maintenance of quality characteristics. The quality of sweetcorn cultivars and their acceptability to consumers is greatly related to their biochemical composition (Evensen and Boyer, 1986). Nowadays, a major determinant of sweetcorn quality is the concentration of sugars (Showalter and Miller, 1962; Varseveld and Baggett, 1980). Other chemical components that are important for the sweetcorn industry and have been related to health include antioxidants such as vitamin C, phenolic compounds and carotenoids (Halvorsen *et al.*, 2002; Chun *et al.*, 2005). However, determination of sugar concentration in supersweet sweetcorn cultivars provides the most important factor for quality control procedures.

Panel perception of sensory attributes is strongly associated with chemical and physical characteristics of kernels (Azanza *et al.*, 1996a) and therefore consumer preference. After the introduction of supersweet sweetcorn to the sweetcorn industry, the postharvest life of this product has been extended to up to 3 weeks. In contrast, normal sweetcorn can be maintained for 5-10 days (Riad and Brecht, 2001). One of the main goals of postharvest fruit and vegetable research is to minimise the losses from the time of production to consumption, whilst maintaining quality (Kader, 2003). The reason that sweetcorn is such a perishable product is its extremely high respiration rate (Kader, 2002). As it has been shown that consumer preference for sweetcorn flavour is strongly related with the concentration of sugars, lots of research has been carried out to increase sugar content through breeding. Nowadays, supersweet sweetcorn includes the

‘*shrunk-2*’ (*sh2*) gene which results in greater concentration and longer maintenance of sugar content, satisfying consumer preferences.

Currently, total sugars and especially the concentration of sucrose in addition to texture-related characteristics are used in the sweetcorn industry as an index of quality status. Rapid and accurate methods are available for this purpose. Sugar content is commonly measured by HPLC (Conrad and Palmer, 1976; Ball and Wetzel, 1977; Dunmire and Otto, 1979; Hurst *et al.*, 1979), while textural characteristics of sweetcorn are usually subjectively or mechanically characterised using sensory tests or with testing machines, respectively. Furthermore, other characteristics such as aroma are also very important in the sweetcorn industry and are said to influence overall consumer acceptability. In particular, ethanol, methanethiol, acetaldehyde, dimethyl sulphide and hydrogen sulphide have been considered as potential compounds whose interaction influences aroma (Flora and Wiley, 1974; Buttery *et al.*, 1994).

The success of the UK sweetcorn industry relies on consumer satisfaction involving increased sales, profit, and quality of sweetcorn corresponding to its value. Therefore, a better understanding of the deficiencies of postharvest handling and storage conditions and of the methods involved to measure quality parameters of sweetcorn would lead to minimisation of quality loss and reduced consumer complaints. In turn, identification/ elimination of these deficiencies would allow improved promotion of the cvs. produced and/or imported into the UK market.

## **1.2 Aims and objectives**

### **1.2.1 Aim**

The current work is funded by Barfoots of Botley Ltd. and Cranfield University and aimed to provide a greater understanding of biochemical and textural changes of sweetcorn cultivars occurring after harvesting. Furthermore, this PhD project aimed to determine the best storage conditions for sweetcorn cultivars in terms of maintaining texture and taste. The overall purpose of the project was to create a better understanding

of the temporal biochemical profile of sweetcorn after harvest and to assess the intrinsic variance of different sweetcorn cultivars as affected by several factors.

### 1.2.2 Objectives

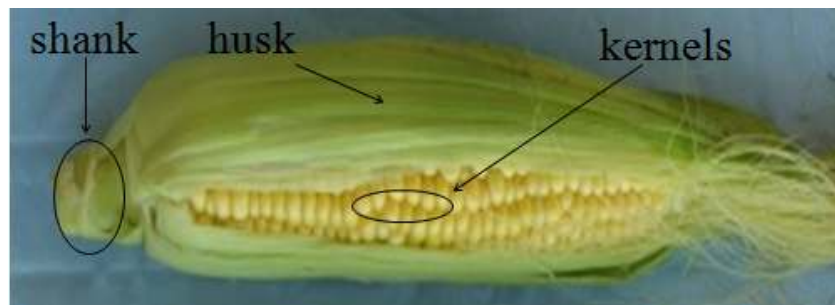
The major quality parameters which have been most frequently studied are sugar content and texture. Thus, sugar content and texture were evaluated in all the experiments accomplished. After the identification of the major quality characteristics of sweetcorn it was considered important to identify the most frequent factors of variation in quality attributes of sweetcorn according to the literature. The most frequent factors found to be the source of variation in the quality characteristics of sweetcorn were: cultivar, storage conditions during transportation, origin, storage packaging and storage temperature. After finding the deficiencies in the literature, the current project aimed to further investigate these factors. The objectives of this thesis are reported below and are better described in the flow charts displayed in Figure 1.2 and 1.3; where the number of objective corresponds to the numbered objectives which are listed below. Objective 1 applies to all chapters, as the sweetcorn cobs of all the experiments were used for that purpose. It was considered more appropriate to include two flow charts as sweetcorn is stored as fresh (Figure 1.2) but is consumed cooked (Figure 1.3). More detail about the chapters referred to in Figure 1.2 and Figure 1.3 are provided in the next Section (Thesis structure).

#### Objectives

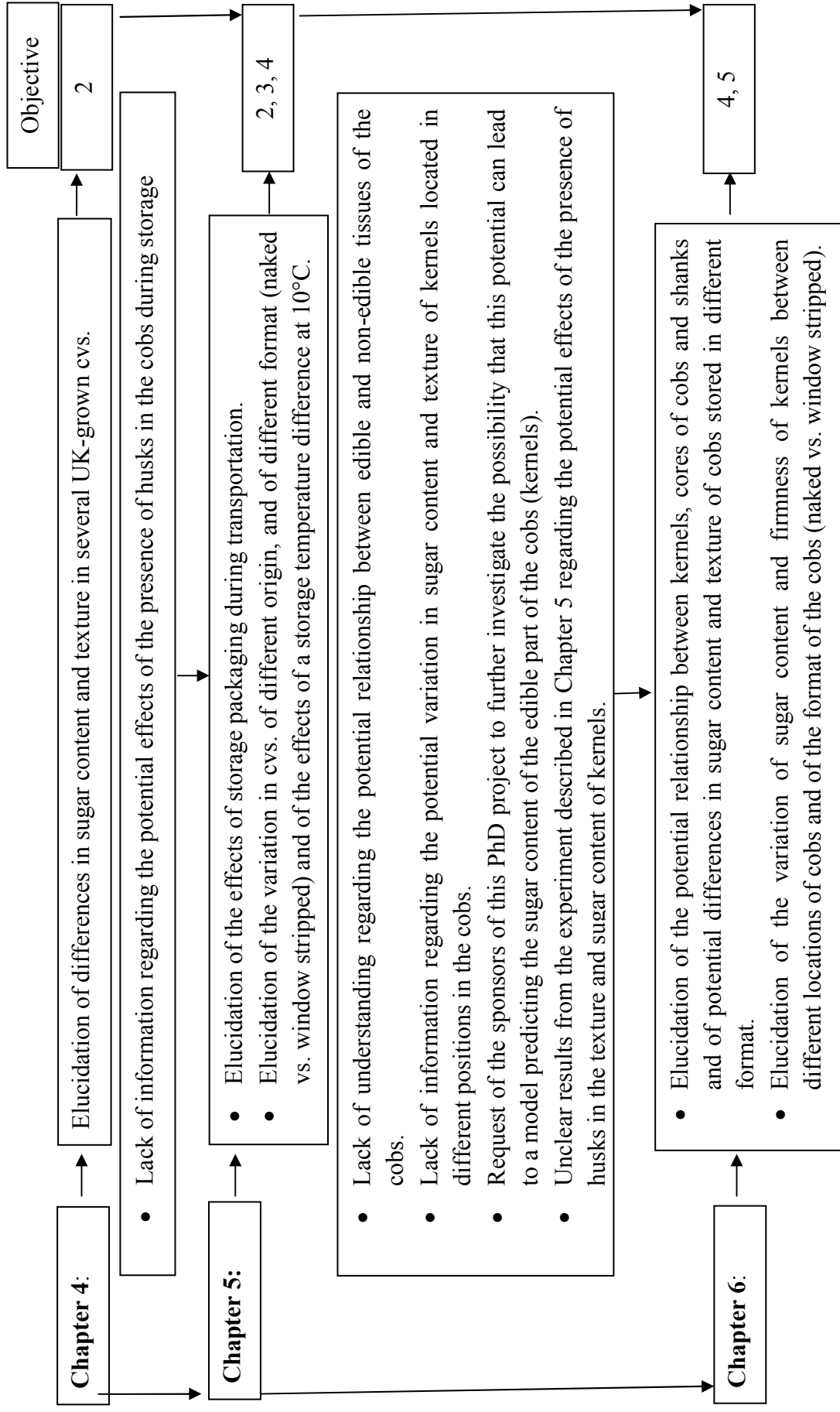
1. To contribute to method development, validation and optimisation of textural characteristics and extraction and quantification of target analytes related to major quality parameters.
2. To investigate the influence of the genotype and the origin of cobs on the texture and sugar content of sweetcorn cobs containing the *sh2* gene.
3. To determine potential differences in the texture and the concentrations of sucrose, glucose and fructose in cobs of sweetcorn cultivars (cvs.) stored at different temperatures.



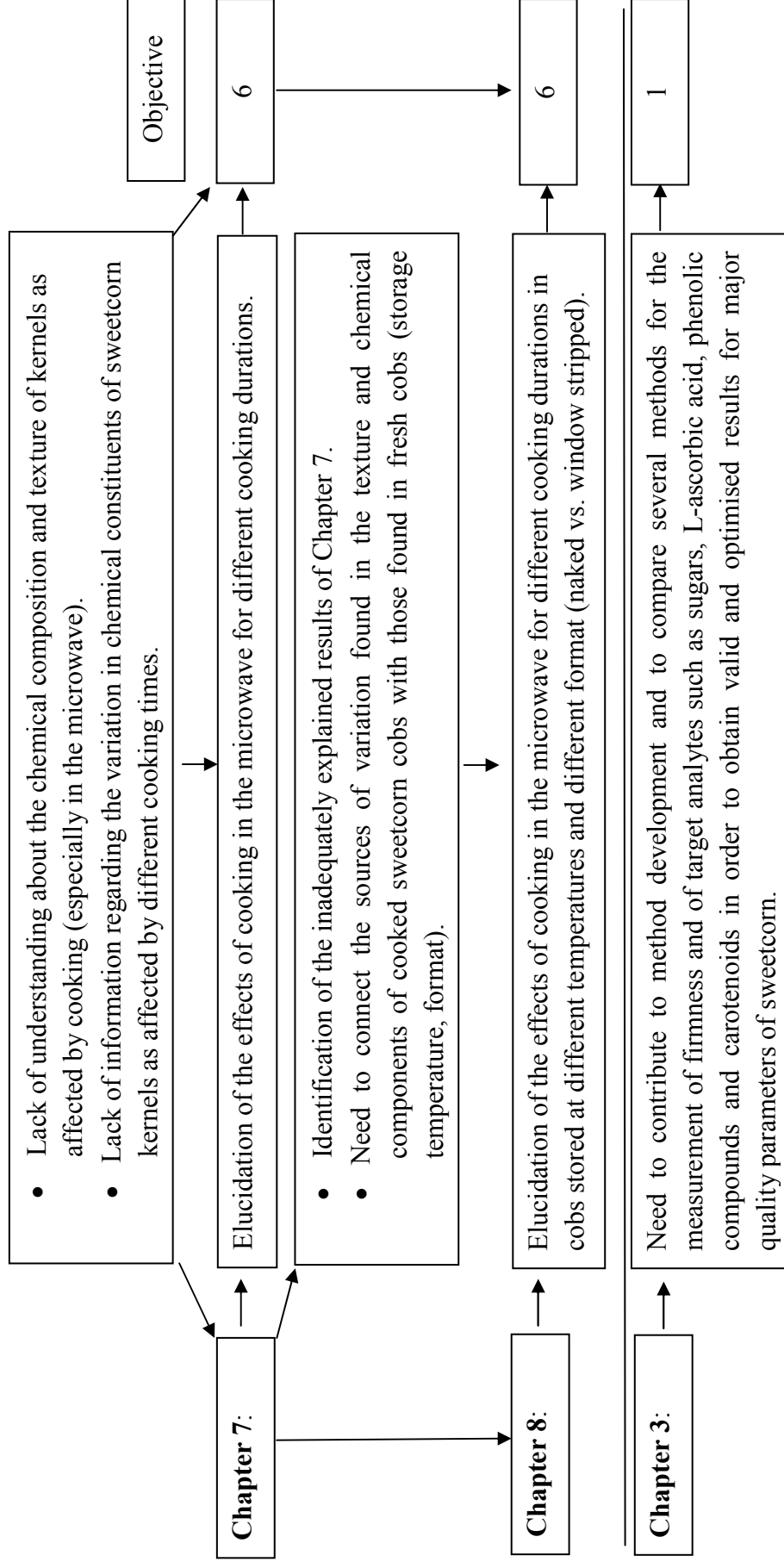
4. To determine differences in texture-related characteristics of sweetcorn cvs. of different format; such as sweetcorn cobs covered with husks vs. cobs without husks. Figure 1.1 displays the part of the cobs named as kernels, husks and shanks.
5. To investigate the spatial, textural and sugar content changes throughout sweetcorn cobs.
6. To elucidate the colour and the nutritional and textural changes occurring under cooking conditions when stored at different temperatures and in different cob formats (with or without husks).



**Figure 1.1:** Shank, husk and kernels of a sweetcorn cob.



**Figure 1.2:** Flow chart (Part A) showing objectives of the experiments described in Chapter 4-6 that concern fresh sweetcorn.



**Figure 1.3:** Flow chart (Part B) showing objectives of the experiments described in Chapter 7-8 that concern cooked sweetcorn and of Chapter 3 that concern validation and optimisation of the methods used for the measurement of quality parameters in sweetcorn.

### 1.3 Thesis structure

This thesis is organised in nine chapters. The second Chapter aims to provide the required literature review, giving information about the sweetcorn industry worldwide, quality parameters of sweetcorn and preharvest and postharvest factors that influence the postharvest life of sweetcorn. Background information about the chemical composition of sweetcorn cultivars during their postharvest life, preferred storage atmosphere environment and sweetcorn properties concerning consumer preferences is also given.

Chapter 3 is the materials and methods used for this project, including physiological and biochemical measurements *viz.* colour, concentrations of CO<sub>2</sub> and O<sub>2</sub>, firmness, sugars, L-ascorbic acid, total phenolic compounds, ferulic acid, carotenoids and total antioxidant capacity. Controlled atmosphere (CA) conditions with beneficial effects in postharvest life of sweetcorn have been investigated. However, in Chapter 4 (Experiment 1) the effects of CA (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) on the sugar and textural profile of seven UK-grown supersweet sweetcorn cultivars were tested, as in the packed sweetcorn industry these concentrations of CO<sub>2</sub> and O<sub>2</sub> are believed to be achievable through the relevant packaging. Results from this work have been published as follows:

- Smyrniotaki, M., Landahl, S. and Terry, L. A. (2009). Temporal changes in textural and taste-related characteristics of seven UK-grown *sh2* sweetcorn cultivars stored under controlled atmosphere conditions. In: *10<sup>th</sup> Controlled and modified atmosphere research conference*, 4-7 April, 2009, Antalya, Turkey. Oral Presentation. (See Appendix C).

The effects of transportation and packaging on the sugar content of sweetcorn cobs (Experiment 2) and the effects of storage temperature, storage format (with or without husks) and of the origin of a supersweet sweetcorn cv. (Experiment 3) are described in Chapter 5. The influence of the format of the cobs during storage in sugar content and texture of kernels was not clearly shown in the results described in Chapter 5. Thus, it was decided to further investigate the potential effects of this factor in several

quality attributes of sweetcorn and specifically in the experiments described in Chapter 6 and Chapter 8.

The effects of genotype and other factors that might influence the textural characteristics of sweetcorn kernels and the chemical composition of kernels, core and shank have been investigated. However, postharvest factors and/or interaction of these factors have not been investigated extensively. Chapter 6 presents results from two experiments that examine the effects that genotype, time of storage, length of shank, format of the cob (with or without husks) and the interaction that these factors might have in the texture and the sugar profile of sweetcorn. Results from this work have been published as follows:

- Smyrniotaki, M. and Terry, L.A. Textural Characteristics and Spatial Sugar Profile of a Super Sweetcorn Stored with or Without Husks. 28th International Horticultural Congress, 22-27 August 2010, Lisbon, Portugal. Poster Presentation. (See Appendix C).

The literature about cooked sweetcorn in the microwave for several cooking durations and subsequent effects of storage time is inadequate. Thus, Chapter 7 refers to the experiment 6 which aimed to elucidate textural and nutritional changes occurring as a result of microwave cooking followed by storage over a period of 10 days. Results of Chapter 7, lead to the experiment described in Chapter 8, which differentiated from the previous one, in further investigation of textural and nutritional characteristics of sweetcorn cobs cooked in the microwave but in this Chapter is also investigated the influence of the storage temperature and the format of the cobs (husks vs. without husks). This work has yet to be published.

Chapter 9 is a general discussion and conclusions of the work considering optimum storage conditions and leading to recommendations for future work. Furthermore, Chapter 9 refers to potential implications of these conclusions for handling and storage at industry, market and home. References are presented in Chapter 10 and Appendices in Chapter 11.

## 2. CHAPTER TWO

### Literature Review

#### 2.1 Sweetcorn morphology, growth and development

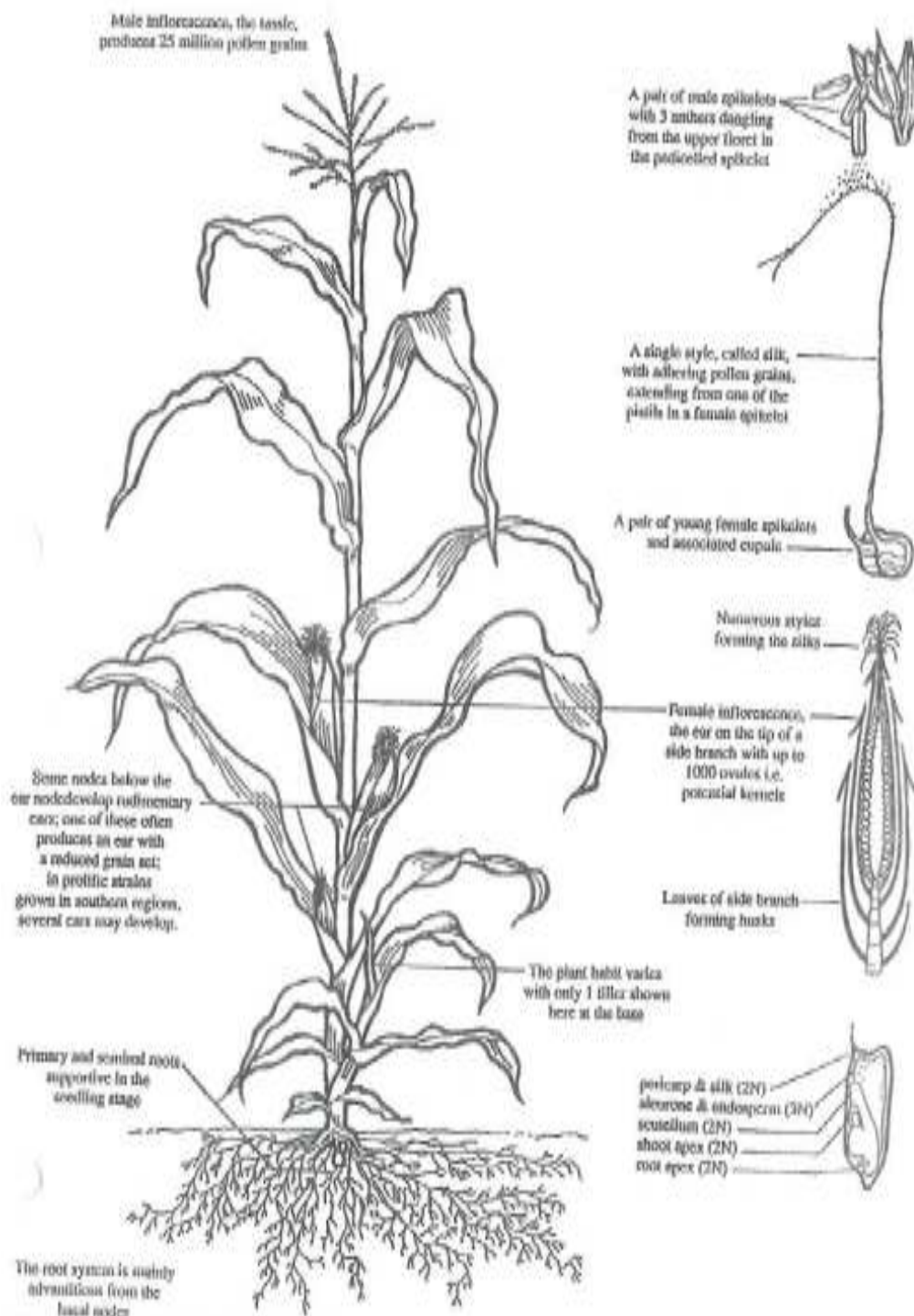
Sweetcorn is a monocotyledonous, herbaceous, monoecious, with separate female (ears) and male organs (tassels), annual seed producing plant of the Poaceae family, with a deep, fibrous root system (Farnham *et al.*, 2003). The corn plant has nodes in the stalk and each one has a single leaf whose sheath surrounds the stalk. In inflorescences that are formed in stem, are the spikelets with the flowers enclosed by two bracts, the lemma and the palet (Kiesselbach, 1949). The morphology of maize is displayed in Figure 2.1.

When the seedling is still small, branches and tassels (staminate flowers), are formed in the stem. Other branches become tillers and other ear shoots (pistillate flowers). Spikelets and subsequently ear shoots can also be formed in branches. Ears are held in the thick stem called the shank (Hipp, 2004) and are positioned centrally to the spike surrounded by husks (Ghorpade *et al.*, 1998). Pistillate inflorescences produce corn ears and spikelets which are actually sessile and produce seeds (Kiesselbach, 1949).

Sweetcorn is frost-sensitive and is usually classified as early, medium and late season, depending on the duration required to produce edible cobs (Ghorpade *et al.* 1998). Development of the corn seed to plant takes a few weeks. The growth period is strongly dependent on variety and location of production, while germination time depends on moisture and soil temperature (Farnham *et al.*, 2003). Variety and regional adaptation affects the size of corn plants (Kiesselbach, 1949).

Tassel emergence indicates that the plant has reached full height. Tassels produce the pollen, which during anthesis is transferred by the wind and germinates on the silks to produce sweetcorn cobs. The edible parts of the sweetcorn cobs are the kernels which briefly consist of the endosperm, the aleurone (a layer under the pericarp), the embryo and the pericarp that encloses them. Kernel characteristics are the

criteria for the classification of corn to popcorn, dent, flour, flint and sweet corn (Farnham *et al.*, 2003). The immature fruit of sweet corn is approximately harvested 18-21 days after pollination, also known as milk stage (Ghorpade, *et al.* 1998).



**Figure 2.1:** Maize morphology (Ghorpade *et al.*, 1998)

## 2.2 Sweetcorn industry

*Zea mays* L. is a popular and essential crop for the agricultural economy worldwide, especially in United States and Canada. Consumption of sweetcorn is continuously increasing in Asia and Europe (Schulteis, 2007). Supersweet sweetcorn (*sh2* corn) is preferred over sugary corns in most of the markets as the amount of proteins and sugars is greater and calories lower (Goldman and Tracy, 1994). Furthermore, the long shelf life and harvest period of supersweets, have established them as preferred type of sweetcorn and thus, in most places have replaced other genotypes in the market such as brittle and sugary enhanced corns (Tracy, 1997).

Sweetcorn is sold in the market as fresh, frozen or canned. Lately, in some countries sweetcorn is also sold in other forms such as soups and fresh-cut sweetcorn kernels even if the last form is problematic due to colour discolouration after cooking (Riad and Brecht, 2001). In 2004, the UK was one of the top five frozen and canned sweetcorn importing countries (Lertrat and Pulam, 2007). UK consumers prefer to import fresh sweetcorn from the United States, France, Thailand, and Canada and recently from other locations such as Senegal due to high quality of the products originating from these regions (Salunkhe *et al.*, 1998). Table 2.1 refers to countries with the greatest sweetcorn import or export value according to data provided by USDA.

**Table 2.1:** Export and import value (1,000 U.S. dollars) of sweetcorn (USDA, 2010)

Countries	Import value	Countries	Export value
USA	39,037	Canada	76,434
France	13,775	United Kingdom	73,285
Italy	12,478	USA	17,673
Spain	8,856	France	10,082
Israel	3,915	Germany	4,052

While small amounts of fresh sweetcorn are grown and consumed as ‘corn on the cob’ in UK, there is no production of canned corn and thus the UK imports it. The



advantages of canned sweetcorn over fresh sweetcorn are mainly the smaller pack sizes and their lower prices. USDA estimations for major production countries of sweetcorn are shown in Table 2.2.

**Table 2.2:** Sweetcorn annual production in metric tonnes (MT), (USDA, 2008)

<b>Countries</b>	<b>2004</b>	<b>2005</b>	<b>2006</b>	<b>2007</b>	<b>2008</b>
USA	3,957,510	4,105,860	4,102,810	3,921,400	3,888,080
Mexico	589,615	627,279	648,238	585,596	610,593
Nigeria	576,000	576,500	577,000	579,000	579,000
France	521,460	496,245	464,264	521,916	521,916
Hungary	508,039	354,210	513,326	514,000	514,000
Peru	377,904	351,341	360,600	332,255	332,255
Indonesia	280,000	313,000	290,000	332,000	332,000
South Africa	320,000	320,000	310,000	310,000	310,000
Thailand	305,000	273,000	305,000	305,000	305,000
Guinea	260,000	260,000	270,000	280,000	285,000
Japan	265,600	250,900	231,400	240,000	240,000
Canada	286,924	252,345	242,663	296,245	216,826
Chile	252,000	230,000	200,000	165,000	165,000
Papua New Guinea	235,000	235,000	235,000	235,000	235,000
New Zealand	112,000	96,500	100,000	90,000	90,000
Australia	40,000	40,000	40,000	42,000	42,000
China	13,310	14,000	33,010	36,010	38,010
Bolivia	31,172	32,160	34,321	34,852	34,852
El Salvador	0	24,780	28,248	30,000	33,305

## 2.3 Quality parameters of fresh sweetcorn

There are several parameters that determine fresh sweetcorn quality. Physical and chemical properties of sweetcorn are influenced by genotype, agrotechnical

procedures and postharvest handling (Felczynski *et al.*, 1999). The major quality characteristic of sweetcorn is sugar content and therefore kernel sweetness (Wann *et al.* 1971; Evensen and Boyer, 1986). Among sugars in sweetcorn, sucrose is the most important, as it predominates significantly in comparison to other sugars (Ferguson *et al.*, 1979; Carey *et al.*, 1982b; Zhu, *et al.*, 1992).

The quality of traditional sweetcorn hybrids decreases after harvesting as a result of moisture loss. The conversion of sugars to starch also contributes to quality loss of sugary sweetcorn hybrids. Thus, as mentioned previously, endosperm mutations have been utilised in order to improve sweetcorn quality. In particular; sugary enhancer and shrunken2 (*sh2*) mutants are of great interest to the sweetcorn industry (Juvik *et al.*, 2003). As endosperm mutated hybrids can have up to three times higher sugar content (Carey *et al.*, 1984; Olsen *et al.*, 1990; Douglass *et al.*, 1993) water content is maintained for a longer time after harvesting (Garwood *et al.*, 1976; Carey *et al.*, 1982b), their quality is greater than standard corn. The positive correlation between moisture and sugar content has been observed in sweetcorn kernels by Azanza *et al.*, (1996b). However, the authors did not explain why moisture and sugar content are correlated which can be explained by the properties of sugars (hygroscopicity, solubility and viscosity). In a review of functionality of sugars (Davis, 1995), it is explained that due to the sugar properties previously mentioned, sugars are associated with water flow control due to cohesiveness and the hydration properties of sugars. In more detail, sugars may restrict starch gelatinisation, resulting in starch hydration and hence, increased moisture content. Further details for sweetcorn mutations follow in section 2.4.1.

The main factors affecting quality of both market (fresh) and processed sweetcorn are reported in Table 2.3. According to this table, Rank factor 1 is highest and it accounts for flavour and texture-related factors as factors of greater significance for the quality of fresh sweetcorn than appearance-related factors. It is also indicated that sweetness of fresh sweetcorn cobs is the most important flavour-related factor. Furthermore, sweetness and kernel texture appear to be more important quality factors for fresh than for processed sweetcorn, while the appearance-related factors are greater in number for fresh sweetcorn.

**Table 2.3:** Factors affecting sweetcorn quality (Ghorpade *et al.*, 1998)

Texture-related factors		Flavour-related factors		Appearance-related factors	
Market	Processed	Market	Processed	Market	Processed
2.6	2.1 Tenderness	2.2	3.0 Sweetness	4.8	3.6 Kernel size
3.5	3.1 Pericarp	3.9	3.3 Flavour	4.9	3.8 Kernel colour
3.6	3.8 Texture	5.1	5.4 Mouthfeel	5.2	Colour of husk
			5.7 Aroma	5.3	Size of ear
				6.1	Insect damage
				6.2	Shank length
				6.0	Size of flag leaves
				6.0	Colour of flag leaves

Rank Factor 1 is highest

Texture and flavour are important quality parameters of sweetcorn related to moisture content and Water Soluble Polysaccharides (WSP) (Wann *et al.*, 1971; Wiley, 1985). Sugar content and texture depends on the type and variety of the corn, the ripeness at harvesting time and the treatments during postharvest life. The creamy texture of sweetcorn kernels is related to the polysaccharide phytyglycogen. The pericarp of *sh2* corns usually has a more crunchy texture than kernels of the other two common types of corn (Ghorpade *et al.*, 1998; Tracy *et al.*, 1993). Tenderness of the pericarp is also a very important quality factor of sweetcorn in terms of texture (Kramer *et al.* 1949; Showalter *et al.*, 1955). According to an old study (Bailey and Bailey, 1938) the thickness and the amount of tissue of the pericarp influenced pericarp tenderness.

Flavour as well as aroma of sweetcorn is more difficult to define compared with texture and sugar concentration. It has been suggested that both flavour and aroma are related to dimethyl sulphide. The greenness of the husks, the freshness of the silks, the uniformity and the turgidity of the kernels also are important quality parameters related to the appearance of sweetcorn cobs (Wiley, 1985).

Appearance and moisture content of the kernels are also used to describe sweetcorn quality and, in addition, maturity. Appearance of kernels in terms of length, width, thickness, geometric mean diameter, sphericity, volume, surface area, colour and

thousand kernel weights is correlated positively with moisture content (Karababa and Coskuner, 2007).

## **2.4 Chemical composition of sweetcorn**

### **2.4.1 Carbohydrates**

#### **2.4.1.1 Sweetcorn cultivars and genetic improvement of their sugar content**

In recent times, new mutants have been used to improve sweetcorn taste and quality. This improvement was approached through altering the carbohydrate profile; the main constituents of sweetcorn total solids. Carbohydrate deposition in endosperm can be modified by several genes (*wx1*, *su1*, *su2*, *sh1*, *sh2*, *sh4*, *ae1*, *dl*, *btl*, *btl2*) (Laughnan, 1953; Garwood *et al.*, 1976; Courter *et al.*, 1988; Watson, 2003).

One of the most popular endosperm mutants as a result of the mutation of field corn is the normal sugary sweetcorn (*su*), which involves alteration of kernel composition. Sugar levels in the endosperm of *su*-sweetcorn are higher than in the endosperm of the wild type corn. Some varieties of normal sugary sweetcorn are the Silver Queen, Jubilee and Gold Cup (Tracy *et al.*, 1993; Ghorpade *et al.*, 1998).

Sugary enhanced (*se*) gene is another common mutant of sweetcorn. This gene, discovered in 1967 by A. M. Rhode, resulted in increased sweetness and tenderness of sweetcorn kernels. This gene also increased maltose content (Tracy *et al.*, 1993; Ghorpade *et al.*, 1998). Varieties that include the *se* gene are called Everlasting Heritage (EH).

Conversion of sugars to starch is slower in *se* sweetcorn compared to *su* type. Thus kernels of *se* sweetcorn cobs can maintain their sweetness for longer than standard sweetcorn, if refrigerated (Tracy *et al.*, 1997; Ghorpade *et al.*, 1998).

For higher levels of sweetness and tenderness, hybrids of one (*se*) parent and one (*su*) parent, are appropriate. These characteristics are even stronger where both parents are (*se*), resulting in *se*<sup>+</sup> varieties, which are fully sugary enhanced. In the last few decades, consumption of shrunken-2 (*sh2*) varieties, also known as ‘Supersweets’ has continuously increased. These varieties are even sweeter than others and furthermore only a small amount of the sugars contained are converted to starch. The mutated *sh2*

gene blocks the conversion of sugars to WSP and starch and consequently sugars are accumulated (Laughnan, 1953; Dickinson and Preiss, 1969). As a consequence supersweets can contain approximately double the amount of sugars as standard sweetcorn (Showalter and Miller, 1962; Courter *et al.*, 1988; Brecht *et al.*, 1990) while phytoglycogen in this genotype is not accumulated (Creech, 1966; Holder *et al.*, 1974). Laughnan (1953) also suggested that sucrose content in *sh2* cultivars is approximately 85% of the total sugars. Common cultivars of this group include: Sucro, Garrison, How sweet it is, Wisconsin natural sweet and Florida staysweet (Ghorpade *et al.*, 1998). A picture of the *sh2*-cultivar Garrison is provided (Figure 2.2).

The combination of 75% *se* and 25% *sh2* genes is called a synergistic variety with main characteristics such as tenderness; inheritance from the *se* gene and sweetness; inheritance from the *sh2* gene (Tracy *et al.*, 1993).

Sugar content of supersweet sweetcorn is even higher in early mature samples than in full mature kernels (Cerning-Beroard and Guilbot, 1975; Gonzales *et al.*, 1976; Hannah and Cantliff, 1976; Reyes *et al.*, 1982). Furthermore, it has been suggested that there is an inverse relationship between the dry weight and sugar content of developing kernels. This relationship indicates that the accumulation of sugars is the result of the incapability of kernels to utilise sugars during their biosynthesis (Doehlert and Kuo, 1994). Thus, this type of sweetcorn is considered important to improve sweetcorn preservation, during transportation over long distances when refrigerated (Olsen and Jordon, 1989), minimising losses.



**Figure 2.2:** *Sh2* sweetcorn cobs of cv. Garrison originated from Senegal (A) and USA (B).

#### 2.4.1.2 Carbohydrates in sweetcorn

Carbohydrates are the major components of maize kernels and the knowledge of their nature and translocation can be very useful in favour of genetic improvement and therefore for alteration of kernel composition which will result in desirable flavour characteristics (Balcony *et al.*, 2007). The major carbohydrates found in sweetcorn are: starch, sucrose (disaccharide), glucose and fructose (monosaccharides). Postharvest change in sucrose concentration, the major sugar compound in *sh2*-sweetcorn, is dependent on storage temperature. At 10°C, loss of sucrose is 10 times faster than at 0°C (Ghorpade *et al.*, 1998). In addition, it has been suggested that during kernel development sucrose might be degraded before entrance into the kernel and then being synthesized again in the tissues. Thus concentration of sucrose depends on the stage of kernel development (Porter *et al.*, 1985; Cobb and Hannah, 1986).

Other subcategories of simple carbohydrates found in maize kernels are sugar alcohols such as myo-inositol (Carey *et al.*, 1982a) and sorbitol (Carey *et al.*, 1982b). In addition, the hexaphosphoric ester of myo-inositol (phytate) was found (O' Dell *et al.*, 1972). Other carbohydrates found are: maltose and some other higher oligosaccharides such as the trisaccharide raffinose, but in very low levels (Inglett, 1970).

Complex carbohydrates can be categorised as structural and storage carbohydrates. Corn plants contain starch which is a major energy storage (non-structural) polysaccharide and some water-soluble polysaccharides (Boyer and Shannon, 1983). A very important water-soluble polysaccharide (WSP) is phytoglycogen (Greenwood and DasGupta, 1958) which is synthesised and stored in the amyloplast (Boyer *et al.*, 1977).

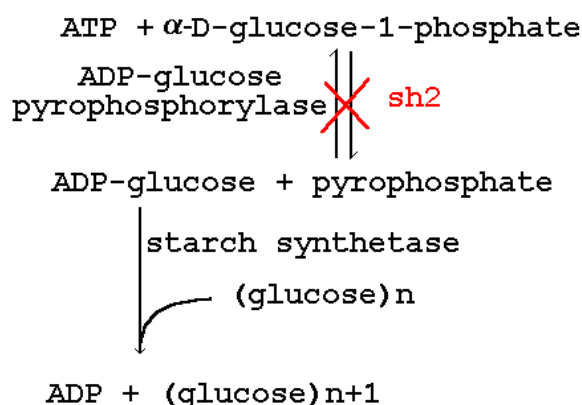
#### 2.4.1.3 Flow of carbohydrates

There are four major tissues involved with the distribution of carbohydrates of maize kernels: endosperm, embryo, pericarp and tip cap. Among carbohydrates, starch which is synthesised in amyloplasts, exists in the highest concentration in kernel tissues and mostly in the endosperm. On the other hand, sugars are found mostly in the embryo (Boyer and Shannon, 1983; Watson, 2003). Carbohydrates move from the phloem into

and throughout the pedicel parenchyma cells via plasmodesmata and then into the apoplasm of the pedicel parenchyma and the placento-chalazal tissue, where inversion of sucrose to glucose and fructose takes place. It should be noted that sucrose is the major translocated sugar. Sugars are then absorbed by the basal endosperm transfer cells (Porter *et al.*, 1985; Griffith *et al.*, 1987). It is believed that sucrose is moved passively into the apoplasm, however it is not well established how basal endosperm cells uptake sugars (Porter *et al.*, 1985). It has also been proposed that the capacity of sugar utilisation may affect the translocation of sugars into the endosperm (Griffith *et al.*, 1987).

#### 2.4.1.4 Importance of adenosine 5'-diphosphate glucose pyrophosphorylase (ADPG) in sweetcorn

ADPG is an enzyme, product of the *sh2* allele and is responsible for starch synthesis (Coe *et al.*, 1988). The *sh2* mutation results in accumulation of sugars at the expense of starch (Tracy, 1997). In particular, the *sh2* gene encodes the large subunits of ADPG. In turn; ADPG converts ADP-glucose into glucose-1-phosphate, which is the substrate for starch synthases (Bhave *et al.*, 1990). As ADPG is regulated by 3-phosphoglycerate and Pi, the modification of its allosteric effector sites allows control of starch yield (Stark *et al.*, 1992). The conversions that ADPG catalyzes are shown in Figure 2.3. It has been found that the enzyme ADPG is relatively low in the presence of the *sh2* gene (Dickinson *et al.*, 1983).



**Figure 2.3:** Schematic diagram of adenosine 5'-diphosphate glucose pyrophosphorylase (ADPG) conversions (Purdue University, 2009)

Lack of ADPG in combination with the presence of WSP and starch causes the increase of sugar content in kernels. Hence, the deficiency of this enzyme results in the accumulation of sucrose at the expense of WSP and starch, which in turn results in a very sweet taste (Boyer and Shannon, 1983). Failure of ADPG production, not only results in accumulation of sucrose but also in aggregation of lipids, instead of WSP and starch (Coe *et al.*, 1988).

## 2.4.2 Phenolic compounds

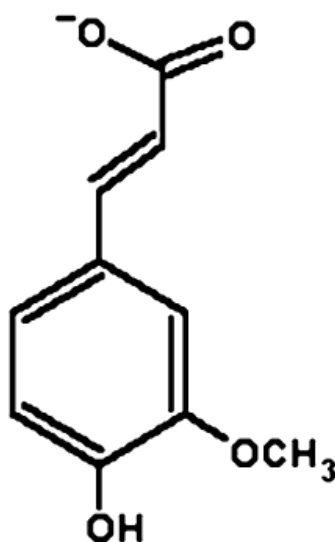
Phenolic compounds are phytochemicals resulting from secondary metabolism of plants and can be structural constituents of the cell walls, or non-structural compounds that are formed under conditions of stress caused by biotic and abiotic factors (Dixon and Paiva, 1995). Phenolic compounds are derivatives of the phenylpropanoid pathway. The most abundant derivative of the phenylpropanoid pathway is lignin, while anthocyanins and phenolic acids contribute to 15.5% of the total compounds synthesised (Kliebenstein, 2004). Phenolic compounds are of special interest due to antioxidant and antimutagenic health benefiting properties; however phenolics are not considered nutrients (Setchell and Aedin, 1999). Furthermore, some phenolic compounds are potentially inhibitors of cell wall oxidative damage resulting from free radicals and reactive oxygen species (Wang *et al.*, 1996) and may offer protection against some degenerative diseases (Hertog *et al.*, 1994).

Phenolic compounds have been reported as a potential reason for browning after cooking due to polymerisation of polyphenolic compounds which is caused by auto-oxidation of phenolic compounds (Talcott and Howard, 1999). Wounding can cause an increase in total soluble phenolics (Babic *et al.*, 1993), while cooking results in a decrease (Riad *et al.*, 2003). Ferulic acid is of special interest for several researchers (Pedreschi and Cisneros-Zevallos, 2007; Lopez-Martinez *et al.*, 2009) as it is the most abundant. Hence, ferulic acid can be used as a marker of treatments influencing phenol content in sweetcorn (Dewanto *et al.*, 2002). The chemical structure of ferulic acid is shown in Figure 2.4.

Ferulic acid is the product of tyrosine and phenylalanine metabolism and is linked to human health such as therapeutic effects against several diseases. In



particular, ferulic acid has been reported to protect against diabetes, cardiovascular disease, cancer and many other diseases (Srinivasan *et al.*, 2007). Other phenolic compounds, apart from ferulic acid, found in yellow sweetcorn are: *p*-hydroxybenzoic acid, protocatechuic, caffeic, sinapic, vanillic, syringic and *p*-coumaric acids, quercetin, kaempferol and 3, 7-di-*O*- methylquercetin-5-glucoside (Kirby and Styles, 1970; Hedin and Callahan, 1990; Shahidi and Naczk, 1995; Dewanto *et al.*, 2002).



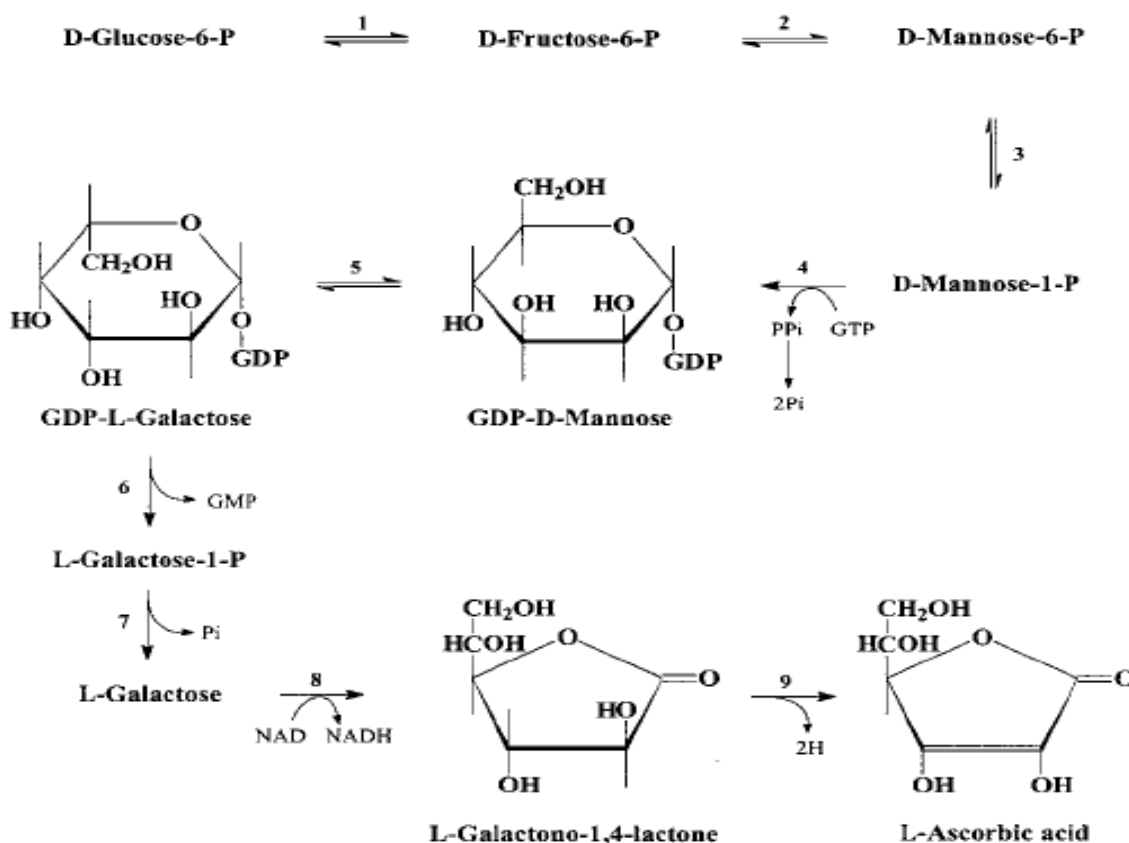
**Figure 2.4:** Structure of ferulic acid (Srinivasan *et al.*, 2007)

### 2.4.3 Vitamin C (L-ascorbic acid)

Vitamin C is a naturally occurring valuable nutrient, yet it is not synthesised by humans. L-ascorbic acid has not only an important role in photosynthesis and photoprotection of plants (Smirnoff, 2000), but also antioxidant and therapeutic properties and therefore contributes to product quality. In more detail, vitamin C has a protective role against free radicals and reactive oxygen species and therefore is beneficial to the human body defence system (Dewanto *et al.*, 2002; Okiei *et al.*, 2009). Smirnoff (1996) also reported other functions of ascorbic acid such as electron transfer, enzyme co-factor and synthesis of oxalate and tartarate. Thus, ascorbic acid can be used

to show antioxidant capacity in foods expressed as vitamin C equivalent antioxidant capacity (VCEAC) (Kim *et al.*, 2002).

A possible pathway of L-ascorbic acid biosynthesis in higher plants is shown in Figure 2.5.



**Figure 2.5:** Suggested pathway of L-ascorbic acid biosynthesis in plants

Source: Wheeler *et al.*, 1998

The bioactive form of vitamin C (L-ascorbic acid) is labile and influenced by several factors such as pH, water content, heavy metal ions ( $\text{Cu}^{2+}$  and  $\text{Fe}^{3+}$ ), packaging and temperature (Nunes *et al.*, 1998; Dewanto *et al.*, 2002; Salkic *et al.*, 2007). In more detail, oxidation of ascorbic acid to dehydroascorbic acid (DHAA) and polymerisation to several inactive products results in degradation and therefore loss of ascorbic acid (Gregory, 1996). Biological activity is not lost under oxidation because this process is reversible. However, when 2, 3-diketoglutaric acid is formed, the process is not reversible (Salkic *et al.*, 2007). Due to the instability of L-ascorbic acid in the presence

of the previously mentioned factors, analysis of L-ascorbic acid can prove unreliable and inaccurate.

#### 2.4.4 Proteins, lipids, fatty acids vitamins and minerals occurring in sweetcorn

Linoleic, palmitic and oleic fatty acid are the most abundant fatty acids present in sweetcorn, but other acids also occur in very low levels (palmitoleic, arachidic, lauric, erucic and other acids) (White and Weber, 2002). Deposition of lipids on the starch surface or within starch granules of kernels does not necessarily stop at the end of starch and protein synthesis. Depending on the type of sweetcorn, the oil content ranges between 5.2-18.4%, whilst protein content is from 2.86 to 3.70%. Vitamins such as niacin, vitamin B6, riboflavin, thiamin and vitamin A and minerals such as Cu, Ni, Zn, Na, Mo and K, also contribute to the chemical composition of sweetcorn. Minerals in kernels occur mostly in the germ; however some of them also occur in the aleurone and endosperm (Boyer and Shannon, 1983; Ghorpade *et al.*, 1998; Watson, 2003). The chemical constituents of sweetcorn are named in Table 2.4.

**Table 2.4:** Chemical composition of sweetcorn (Source: Salunkhe *et al.*, 1998)

Constituents			
Water	Folic acid	Iron	Valine
Protein	Vitamin C	Copper	Malic acid
Fat	Arginine	Phosphorous	Citric acid
Carbohydrates	Histidine	Boron	Quinic acid
Crude Fibre	Isoleucine	Vitamin E	Succinic acid
Minerals	Leucine	Vitamin B1	Glucose
Sodium	Lysine	Vitamin B2	Fructose
Potassium	Methionine	Nicotinamide	Sucrose
			Total Dietary
Magnesium	Phenylalanine	Pantothenic acid	Fibre
Calcium	Threonine	Vitamin B6	
Manganese	Tryptophan		

Concentration of proteins in the kernels of the corn varies due to several factors such as genotype, type of corn and growing conditions. Protein content of endosperm of the kernels (albumins, globulins, prolamines and glutelins) constitutes approximately  $\frac{3}{4}$  of total protein content of kernels (Lawton and Wilson, 2003). Previous research also suggested that hardness is correlated to the concentration of proteins, and in particular to zein fractions (Holding and Larkins, 2006; Fox and Manley, 2009). It is worth noting that comparison of levels of nutrients such as minerals, carotenoids and fibre indicated that are generally similar to those of fresh and processed products, including sweetcorn (Rickman *et al.*, 2007b).

#### 2.4.5 Carotenoids and tocopherols

Carotenoids are very important yellow-orange and lipid-soluble terpenoid pigments which can act as pro-vitamins and antioxidants and in addition find applications as food colorants (Kopsell *et al.*, 2009; Fernandez-Sevilla, 2010). Some carotenoids are precursors of vitamin A (cryptoxanthins,  $\alpha$ -carotene and  $\beta$ -carotene). Both provitamin A and non-provitamin A (lycopene, zeaxanthin and lutein) carotenoid compounds can scavenge free radicals which contribute to damage of cells, tissues and also DNA which can result in the formation of cancer cells. These compounds can also act as inhibitors of cell proliferation and transformation (Roberfroid, 1995; Kopsell and Kopsell, 2006). Furthermore, cardiovascular and other chronic diseases may be partially prevented due to antioxidant carotenoids (Wolf, 1994; Evangelou *et al.*, 1997).

Carotenoids can be classified as carotenes and xanthophylls (Groff *et al.*, 1995). In particular,  $\alpha$ -carotene,  $\beta$ -carotene and lycopene are hydrocarbons (carotenes); and lutein,  $\beta$ -cryptoxanthin and zeaxanthin are xanthophylls or in other words, oxygenated products of carotenes. Lutein and zeaxanthin are the two major carotenoids in the fresh sweetcorn market while  $\beta$ -carotene,  $\alpha$ -carotene,  $\beta$ -cryptoxanthin and antheraxanthin also occur but in lower amounts (Kurilich and Juvik, 1999b; Kopsell *et al.*, 2009). In particular, lutein and zeaxanthin are deposited in the retina as macular pigments (Bone *et al.*, 1997), filtering UV light and protecting the retina (Kopsell and Kopsell, 2006). These xanthophylls have been reported for their potential ability to reduce the risk of cataract development and macular degeneration of elderly people (Seddon *et al.*, 1994).

Hence, their quantification in sweetcorn is important due to their health promoting properties and colour.

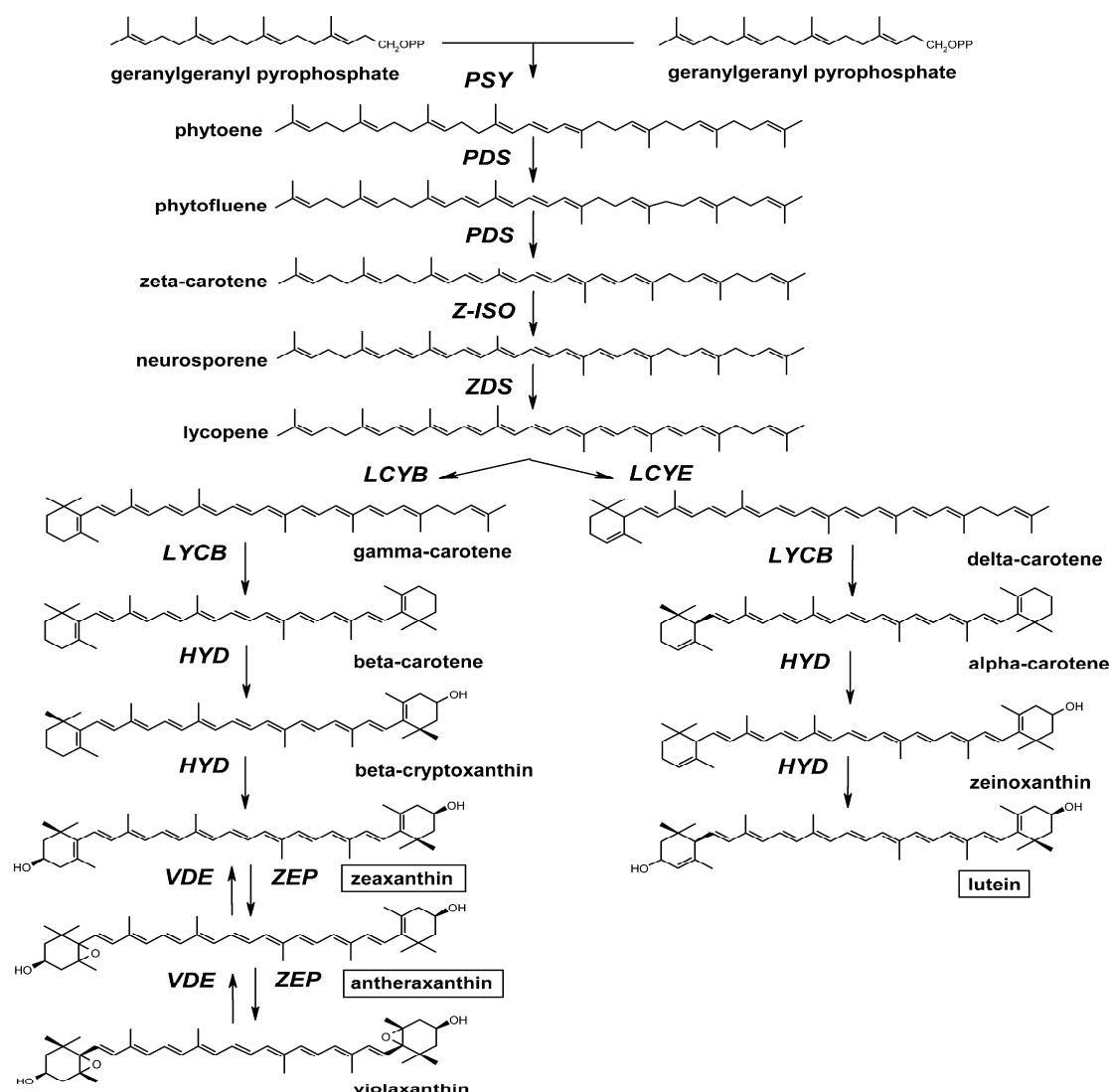
**Table 2.5:** Means  $\mu\text{g/g}$  Dry Weight of carotenoid in kernels of different corn genotypes

Source: Ibrahim and Juvik, 2009

Genotype	Lutein	Zeaxanthin	$\beta$ -cryptoxanthin	Total carotenoids
1645-121 <i>su1</i>	3.7	2.7	0.2	6.2
IL27a <i>su1</i>	0.7	0.5	0.3	1.5
IL451b <i>su1</i>	10.6	3.3	0.5	14.4
IL677a <i>se1</i>	1.7	2.1	0.2	4.0
IL678a <i>se1</i>	2.6	2.6	0.2	5.3
IL731a <i>se1</i>	1.6	2.2	0.3	4.1
IL2027-8 <i>sh2</i>	9.9	4.3	0.7	14.9
IL2027-7 <i>sh2</i>	2.7	1.3	0.0	4.0
La453a <i>sh2</i>	18.3	8.1	1.4	27.8

Carotenoid content in plant tissues is affected by genetic and environmental factors such as temperature, growing conditions and light. Carotenoid bioavailability is affected by postharvest handling and potential processing of these factors can alter their chemical structure. Generally, the carotenoid chemical structure can be altered by biotic and/or abiotic factors which may cause isomerisation, degradation or enzymatic oxidation. The passive absorption of carotenoids by humans occurs only in the presence of fat in the small intestine (Kopsell and Kopsell, 2006).

Other compounds found in sweetcorn kernel tissues are the tocopherols (vitamin E analogues), which are antioxidants with antiproliferative and protective properties against degenerative diseases. Tocopherols occur mostly in the embryo of kernels. Variability in tocopherol content and therefore their potential health promoting properties is strongly dependent on genotype. The primary form of tocopherols has been reported to be the  $\gamma$ - form (Maydani, 1995; Kurilich and Juvik, 1999b).



**Figure 2.6:** Biosynthetic pathway and chemical structures of carotenoid biosynthesis in plants (Kopsell *et al.*, 2009)

Carotenoid content in kernels of different corn genotypes is presented in Table 2.5. Ibrahim and Juvik (2009) concluded that approximately one corn ear of a genotype characterised by high carotenoid content is sufficient for the suggested daily intake of 6 mg of lutein and zeaxanthin. Especially in the *sh2* sweetcorn cvs., lutein concentration was significantly greater than in other mutations (Kurilich and Juvik, 1999b).

When two molecules of geranylgeranyl phosphate are condensed; phytoene is formed, via phytoene synthase and this is the beginning of the biosynthesis of carotenoids. In turn; phytoene desaturase (PDS) and zeta-carotene desaturase, are the

enzymes that form lycopene from phytoene. (Kopsell *et al.*, 2009) (Figure 2.6). Membranes of amyloplasts, chloroplasts and chromoplasts are the locations where the biosynthesis of carotenoids occurs (Gallagher *et al.*, 2004).

#### **2.4.6 Antioxidant capacity**

Antioxidant properties of phytochemical compounds increase the need to further investigate their potential bioactivity which can be measured by several methods. This need relies on the fact that information on the potential bioactivity of such compounds will contribute to enhanced knowledge of their metabolism in biological systems and therefore of their dietary intake. Each method used to measure antioxidant activity has advantages and disadvantages (Prior *et al.*, 2005). Generally, the majority of methods used to measure antioxidant capacity measure the inhibition of radical species by the antioxidants which occur in the samples. Some commonly used methods are: the cellular antioxidant activity (CAC) assay, total oxyradical scavenging capacity (TOSC) assay, ferric reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity methods (TEAC), and the oxygen radical absorbance capacity (ORAC) which have previously been used for quantification of antioxidant capacity in corn (Dewanto *et al.*, 2002; Riad, 2004; Nagah and Seal, 2005; Wolfe and Liu, 2008; Xu *et al.*, 2009).

### **2.5 Texture**

#### **2.5.1 Texture parameters**

Kernel texture is an important sweetcorn characteristic, influencing sweetcorn quality and consumer acceptability and preference. Azanza *et al.* (1996a) determined chemical, physical and sensory sweetcorn characteristics that grouped them in three factors: taste, texture and aroma. Sucrose and starch aroma were included in the factor taste and sweetcorn and grassy aroma as well as concentration of an unknown volatile were included in the factor aroma. Crispness, juiciness and tenderness were used to describe texture. The authors concluded that these kernel characteristics were well correlated with panel perception of sensory characteristics. In particular, they concluded

that texture ranked 30.5% in the overall liking (taste 45.1 and aroma 24.4%). These scores were the contributions of these attributes to the multiple regression models that the authors used for their study. Water soluble polysaccharides content, moisture and tenderness are some of the factors which influence texture (Szymanek *et al.*, 2003). Creaminess and pericarp tenderness are considered very important sweetcorn texture parameters according to consumers (Culpepper and Magoon, 1927). Tenderness (softness) as determined by sensory panels is negatively correlated with the thickness of sweetcorn pericarp (Gould *et al.*, 1951) and is also dependent on genotype and ripeness phase. A previous study also suggested that heritability of pericarp thickness (consumers prefer thin pericarp) and for some specific cvs. was even higher than heritability of soluble solids content (Cardoso *et al.*, 2002).

Kernel texture may also be influenced by the ratio of water soluble starch (phytoglycogen) over insoluble starch (Culpepper and Magoon, 1927). A previous study reported that pericarp tenderness perception of sweetcorn by a taste panel was correlated with penetrometer readings. In particular, it was found that lower readings were given by the penetrometer for sweetcorn cobs harvested at early stages and panellists perception of tenderness showed that early harvested sweetcorn cobs were preferred (Hale *et al.*, 2004). Huelson (1954) suggested that tenderness of kernels can be defined as the predisposition of the pericarp to fragmentation during mastication.

In the past, it was found that according to sensory panels, sweetness is positively correlated with juiciness, and both are negatively correlated with starchiness. Starchiness is also negatively correlated with tenderness (Azanza *et al.*, 1996b). In addition, it has been recorded that temperature is negatively correlated with firmness (Bourne, 1982). In fact, the firmness of a fruit or vegetable strongly depends on its cell walls structure/ structural integrity and is also negatively related to the concentration and activity of cell wall degrading enzymes such as polygalacturonase. Thus, a decline in firmness is accompanied by cell wall thinning and/or attenuation of tissue cohesiveness (Brett and Waldron, 1996). As elevated temperature leads to tissue degradation this therefore affects the firmness of plant tissues. Furthermore, temperature affects the water volume of plant tissues and therefore the cell tension of the tissues and consequently firmness (Chen, 1993). Thus, elevated temperature leads to a decline of water volume in the tissue, decreased cell tension and hence, decreased load force for



the penetration of the tissue (firmness). However, as temperature also negatively affects tissue viscosity of cell walls, the final effect of temperature on firmness also depends on viscosity (Hertog *et al.*, 2004).

### 2.5.2 Texture measuring devices

Measurement of kernel firmness can be strongly dependent on the type of device which has been used (Sprague and Dudley, 1988). There are several types of texture measuring devices with the penetrometer being the most popular of them. Their use is based on the principle of measuring the force required from the probe of the device to penetrate the product to a given depth, or the total depth of penetration, giving a measure of firmness or toughness. The higher the force, or the smaller the penetration depth; the more resistant is the product. On the other hand, compressimeters test the resistance of the product to compression, and measure the force required to produce a given deformation (usually expressed as softness), or the deformation caused by a given force (usually expressed as firmness) (Szczeniak, 1973).

Shearing devices test the force required to shear a specimen, used as a quantitative measure of tenderness, while cutting devices usually provide measurements of work, by a blade or a wire cutting through the samples. In particular, texture of corn kernels has been evaluated by Kramer Shear Cell (Barrett *et al.*, 2000; Yousef and Juvik, 2001). In food studies, cutting devices are mainly used for vegetables that contain fiber such as asparagus. In contrast, masticometers, mimic conditions of mastication. The textural parameters quantified by these devices are: cohesiveness, hardness, adhesiveness and springiness. Apart from several other miscellaneous and multi-purpose units, consistometers are also used to define texture. In more detail, consistometers concern empirical instruments that mainly measure the resistance to a rotating paddle or spindle. Furthermore, in food science rotational and capillary viscometers are used. Last but not least, other common types of texture measurement devices are the extrusion devices. The weight of material extruded, total work done and extrusion time are some of the measured variables (Szczeniak, 1973).

## 2.6 Preharvest factors that affect quality and postharvest life of sweetcorn

The significance of understanding the preharvest factors affecting the postharvest life of sweetcorn is crucial as after harvest its quality cannot be improved since the cob has been detached from its nutritive and water sources. Thus, it is important to know the best possible preharvest factors affecting sweetcorn life in order to have the best possible initial product and to understand how to delay or reduce postharvest deterioration (Hewett, 2006).

An important factor that influences the quality of sweetcorn is the stage of physiological-horticultural maturity that cobs are harvested, which is usually defined from the appearance of the kernels. Presence of kernels located near the tip of the ear and lack of gaps between kernel rows, are indications for matured sweetcorn cob (Brecht *et al.*, 1990). On the other hand, sweetcorn cobs are also considered physiologically mature, when the deposition of dry matter in the kernels and therefore dry matter accumulation has reached its maximum level (Farnham *et al.*, 2003). According to Afuakwa and Crookston (1984), a good indicator of physiological maturity is the removal of the milk line which is actually a border, separating the liquid phase of the endosperm from the solid one, from the crown to the tip of the kernel. The milk line can be easily observed opposite the germ of the kernel. Sweetcorn postharvest life and quality is affected by harvest time. When sweetcorn is harvested at a time when kernels contain a high concentration of sugars, postharvest life of sweetcorn will be extended at the retail level (Wiley *et al.*, 1989). The period of the optimum harvest maturity is very short and after this peak sweetcorn quality declines rapidly. Therefore, optimum sweetcorn quality is achieved when sweetcorn is harvested at the peak of maturity which depends on several factors such as the type of the cultivar, the further processing that sweetcorn may undergo and the moisture content of the kernels (Szymanek, 2009).

Unfortunately, due to the variation in consumer/individual preference and perception, it is quite difficult to define peak sweetcorn quality leading to various sweetcorn cultivars being grown/sold in an attempt to satisfy different purchasers and final recipients (Hewett, 2006). Thus peak sweetcorn quality refers to sweetcorn free of

pathogens, with maximum nutritional value and optimum colour, size, aroma, texture sweetness, flavour and shape of kernels as they are defined by the target market (Azanza *et al.*, 1996a; Hewett, 2006). Apart from the maximum size of kernels, Motes *et al.* (2007), also stated that at optimum harvest maturity (and therefore at peak sweetcorn quality) kernels are ‘plump, sweet, milky, and tender’, while Evensen and Boyer (1986) stated that husks shall be green and kernels shall not have any mechanical damage.

Furthermore, it has been found that appearance characteristics of sweetcorn kernels such as length, thickness, width and volume of kernels increases linearly with increase of moisture content (Karababa and Coşkuner, 2007), while time of harvesting is also an important preharvest factor. In particular, the appropriate time of harvesting is carried out when the respiration rate is at its lowest such as early in the morning or late in the afternoon. However, proper handling in order to reduce the risk of microbial infections is always an important factor that can influence sweetcorn quality (Zagory and Kader, 1988). Microbial infections can be affected by temperature and moisture and their susceptibility to these factors vary between pathogens. Moisture and temperature are interacted and also affect postharvest characteristics of sweetcorn (Farnham *et al.*, 2003). For instance, moisture stress might result in kernel denting, deteriorating the visual quality of sweetcorn. Furthermore, the harvest procedure is also crucial, as inappropriate harvest can increase surface damage of the kernels (Farnham *et al.*, 2003).

Additionally, age, variety and growing conditions of sweetcorn are major preharvest factors influencing product quality. In more details, weather conditions, soil type, agricultural techniques, insecticides, pesticides, water and nutrient supply affect sweetcorn phenotype (Farnham *et al.*, 2003). Especially environmental conditions influence strongly sweetcorn quality, as it is not a crop that is grown in a protected environment (Hewett, 2006). Age of sweetcorn is also defined as a preharvest factor affecting sweetcorn quality with early mature kernels being of higher quality than late mature kernels (Ghorpade, 1998; Farnham *et al.*, 2003).

The genetic material is one of the most important preharvest factors affecting postharvest life of sweetcorn as it predefines the maximum levels of taste, colour, shape, sweetness, texture and other quality attributes that could be achieved. For instance, due to the *sh2* gene, the high sugar content and the longevity of the shelf life

of supersweet sweetcorns, results in better quality for consumption than standard sweetcorn. That is why genetic engineering has been used to improve postharvest life of corn (Hewett, 2006).

Knowing the preharvest factors that affect the postharvest quality and life of sweetcorn is an important tool for discussing the variation between several quality attributes of sweetcorn. Thus, this could probably contribute to further understanding the postharvest changes that are not easily explained by differences/changes in postharvest handling procedures.

## **2.7 Postharvest factors that affect the quality and life of sweetcorn**

Sweetcorn is a perishable product with a high respiration rate that is significantly influenced by storage temperature and the concentrations of O<sub>2</sub> and CO<sub>2</sub> in storage environment (Riad and Brecht, 2001). It has been reported that storage time can also influence the respiration rate of sweetcorn but to a lesser extent than the previously mentioned factors (Morales-Castro *et al.*, 1994). This usually happens due to processing of sweetcorn such as de-husking which results in wounding-mechanical damage and therefore increased respiration (Riad, 2003). Generally, *sh2* sweetcorn can last up to two weeks, under optimum storage conditions (Brecht, 2002).

Thus, storage temperature at low levels but greater than 0°C, shrink wrapping and irradiation before storage, can be considered as appropriate methods for maintaining postharvest quality of sweetcorn (Deak *et al.*, 1987; Türk *et al.*, 2001; Brecht, 2002). Nowadays, according to EU legislation (Directive 1999/3/EC), irradiation is only allowed for selected categories (spices, dried aromatic herbs and vegetable seasonings) in which sweetcorn is not classified (Eur-Lex. Europa.eu, 2010).

For the purpose of this thesis the major postharvest handling conditions and/or procedures (temperature, CA, MAP) are assessed individually. Yet, it was considered important to include a reference to changes occurring in sweetcorn cobs during storage prior assessing the postharvest factors that affect the quality and postharvest life of sweetcorn. In the Table 2.6 below, are stated synoptically the optimum storage conditions for sweetcorn. The optimum atmospheric composition during storage is not included in that Table, but is discussed in Section 2.7.3.

**Table 2.6:** Optimum storage conditions for sweetcorn

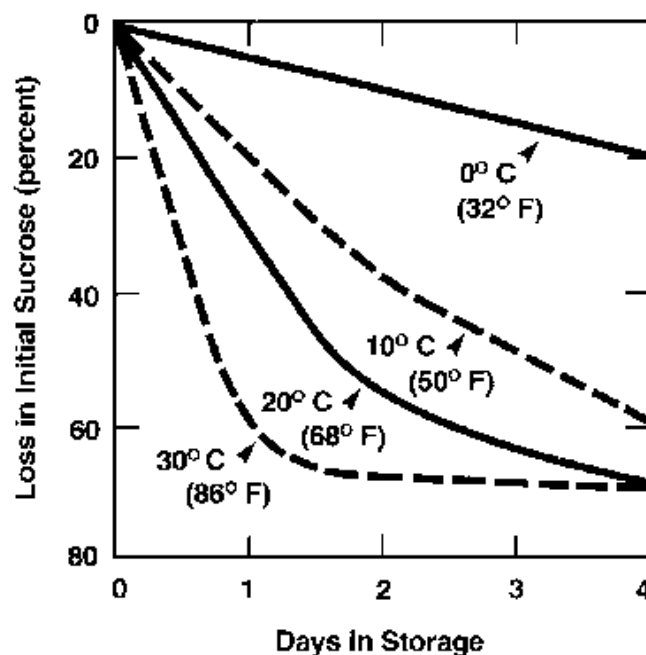
Conditions	Source
Temperature: 0°C	Brecht, 2002
Flag leaves and long shanks: trimmed	Brecht <i>et al.</i> , 1990
High levels of Relative Humidity (90-98%)	Brecht <i>et al.</i> , 1990; Fritz <i>et al.</i> , 2010
Avoid sweetcorn packs in bulk piles as will result in increased heating and respiration	Fritz <i>et al.</i> , 2010

### 2.7.1 Changes occurring in sweetcorn cobs during storage

During the postharvest life of sweetcorn, lots of changes occur, such as loss of taste attributes, discoloration and dehydration of husks, sugar loss, kernel dehydration and denting. These changes contribute to product deterioration and they are related to the high respiration rate that occurs during storage (Wiley, 1985; Hardenburg *et al.*, 1986). Kernel desiccation, dehydration of husks and kernel denting as a result of water loss can subsequently restrict the shelf life of sweetcorn (Showalter, 1967). The development of pathogens is one more factor that makes fresh sweetcorn a perishable product (Saltveit, 2003).

It has been recorded that the limited storage life of several *sh2* sweetcorn varieties at 5°C storage temperature over 9 days, was result of husk dehydration and occasionally of kernel denting (Brecht *et al.*, 1990). The aim of this study was to study the differences among the target characteristics of this study between *sh2* and standard sugary (*su1*) genotypes. The authors recorded sugar levels, % dry weight of pericarp and appearance quality ratings in terms of husk colour, drying and silk and kernel appearance in sweetcorn cobs stored in the conditions mentioned above. However, no comparison of the effects of different storage temperatures was assessed. Brecht *et al.* (1990) also concluded that sweetcorn cobs of the *sh2* genotype had the double concentration and less green colour than cobs of the *su1* genotypes.

In Figure 2.7 it is shown that sucrose content of sweetcorn declines over storage and that the higher the storage temperature the greater is the decline. This Figure also provides useful information regarding the effect of temperature in postharvest life of sweetcorn (Section 2.7.2).



**Figure 2.7:** Sucrose loss of sweetcorn cobs stored at 0, 10, 20 and 30°C during 4 days of storage (Source: USDA handbook No. 66: The Commercial Storage of Fruits, Vegetables, and Nursery Stocks.).

### 2.7.2 Effect of temperature

According to Hardenburg *et al.* (1986), a temperature of 0°C during transportation and storage is recommended. However, temperature is not only important for cold storage but also for precooling, which is a standard method for field heat removal. It has been well documented that high relative humidity (>90%), precooling and storage at low temperatures (0-1°C), are essential for maintaining high postharvest quality of sweetcorn (Lutz and Hardenburg, 1968; Evensen and Boyer, 1986; Geeson *et al.*, 1991; Brecht, 2002). The importance of precooling relies on its beneficial effects against rapid sugar loss after harvest which occurs as a result of high heat generation due to the high respiration rate of sweetcorn (Evensen and Boyer 1986). The respiration

rate of sweetcorn is discussed in the next Section. However, on Table 2.7, respiration rates of sweetcorn depending on storage temperatures are displayed.

**Table 2.7:** Respiration rates of sweetcorn in temperatures ranging from 0 to 25°C (Source: Brecht, 2002).

Temperature	mg CO <sub>2</sub> kg <sup>-1</sup> h <sup>-1</sup>
0°C	30 to 51
5°C	43 to 83
10°C	90 to 120
15°C	142 to 175
20°C	210 to 311
25°C	282 to 435

Sweetness and flavour of *sh2* cultivars decline faster when stored above 4°C (Olsen *et al.*, 1990). It is said that *sh2* sweetcorn, while having greater sugar content than standard *su1* corn, lose their sugars at the same rate. Thus, in only one day, 60 % of the sugar content of both *sh2* and *su1* corns might be converted in starch when the cobs are stored at 30°C and only the 6% when stored 0°C (Brecht *et al.*, 1990). The higher the storage temperature, the more rapid is the deterioration of sweetcorn. The number of days of storage for these hybrids at 4°C for an acceptable quality is approximately 10 days (Olsen *et al.*, 1990).

### 2.7.3 Effect of CA

CA storage is advantageous for the extension of shelf life of perishable vegetables including sweetcorn (Aharoni *et al.*, 1996; Kader *et al.*, 1989). Lots of research has been undertaken in order to identify the ideal gas levels of storage atmosphere for sweetcorn (Chinnan, 1989; Riad *et al.*, 2002; Riad and Brecht., 2003). Reduction of O<sub>2</sub> and/or increase of CO<sub>2</sub> concentration is a method of reducing respiration rate and therefore a helpful tool for modifying atmosphere (Kader *et al.*, 1989; Rodov *et al.*, 2000). Moreover, carbohydrate loss and development of pathogens

is inhibited (Risse and McDonald, 1990). While colour loss due to chlorophyll degradation can also be prevented using controlled atmosphere with increased CO<sub>2</sub> levels (Riad and Brecht, 2003), colour of husks is better maintained at low CO<sub>2</sub> levels (Aharoni *et al.*, 1996). Nevertheless, fermentation and therefore the deterioration of products can occur at very high levels of CO<sub>2</sub> and reduced levels of O<sub>2</sub>. Last but not least, sugar content of sweetcorn which is its major quality parameter is reduced more slowly under higher levels of CO<sub>2</sub> or lower levels of O<sub>2</sub> (Schouten, 1993; Riad *et al.*, 2003).

Some beneficial and detrimental effects of CA and MA are reported in Table 2.8. The irregular ripening of fruits and vegetables, a common problem associated with CA and MA, is not considered as a potential effect on sweetcorn as it undergoes senescence rather than ripening.

Advantageous gas modification is applied in combination with low storage temperature to further improve postharvest quality and shelf life (Deak *et al.*, 1987). Riad and Brecht (2003) compared CA conditions composed of 2% O<sub>2</sub> and 0, 15 or 25% CO<sub>2</sub> against air storage conditions at 5°C storage temperature during a period of 14 days. Their results indicated that the best storage condition in terms of quality preservation was composed of 2% O<sub>2</sub> and 15% CO<sub>2</sub>. The quality preservation was assessed considering the preservation of the initial sugar content of kernels and visual quality characteristics such as husk greenness and kernel appearance. It was also found that the respiration rate of sweetcorn in CA composed of 2 kPa O<sub>2</sub> and 25 kPa CO<sub>2</sub> was higher than in the case of air storage when stored at 5°C and therefore sweetcorn was better preserved in air storage conditions. The same research proposed that the green colour of the husks is maintained better in high levels of CO<sub>2</sub>. While the appearance of the kernels and the silks were better in CA, kernel denting was not affected. Furthermore, it has been reported that CA can prevent the browning of fresh-cut sweetcorn kernels after cooking (Riad *et al.*, 2003).



**Table 2.8:** Beneficial and detrimental effects of CA and MASources: Brecht, 1980; Kader, 1980; Daniels *et al.*, 1985; Kader *et al.*, 1989

Beneficial effects of CA and MA	Disadvantageous effects of CA and MA
Reduce respiration rate	Irregular ripening
Allow handling of chilling sensitive fruits	Susceptibility to decay of storage life
Reduce the incidence of some physiological disorders	Physiological disorders
Decrease susceptibility to postharvest diseases	Activation of some anaerobic pathogens

#### 2.7.4 Effect of MAP

The difference between CA and modified atmosphere (MA) is that CA is created artificially while MA passively (i.e. by the product itself). In addition, CA is monitored and set in specific gas concentrations, while the control of the atmosphere in MAP is less accurate (Kader *et al.*, 1989). In MA conditions, the desirable result is achieved by using appropriate plastic films that permit passive modification of the atmosphere (Chinnan, 1989) allowing a limited degree of control of the gas concentrations existing in the package. Semipermeable plastic film is the most commonly used film in terms of MAP systems (Brecht, 2009). In particular, gaseous permeability is based on nature and thickness of polymer as these factors can influence the rate of diffusion (Brandenburg and Zagory, 2009). This happens due to the respiration of sweetcorn, O<sub>2</sub> level is reduced and CO<sub>2</sub> level is increased as the exchange of air between the inner and the outer environment of the package is reduced (Chinnan, 1989). Consequently, reduced respiration means reduced weight loss and extended shelf life (Kader *et al.*, 1989) while postharvest diseases are inhibited (Daniels *et al.*, 1985). However, it has been reported that compared to polymeric film packaging, perforation-mediated modified atmosphere packaging of sweetcorn can be more beneficial, while polyvinylchloride film can be replaced by polyolefin stretch films (Aharoni *et al.*, 1996; Riad *et al.*, 2002).

Furthermore, respiration can be restricted from MAP, while also prohibiting the anaerobic process in the products and this can be achieved by increasing the concentration of CO<sub>2</sub> or reducing the concentration of O<sub>2</sub> (Chinnan, 1989; Kader *et al.*, 1989).

## 2.8 Effects of cooking on sweetcorn cobs

Sweetcorn is a commodity which is consumed cooked and most commonly grilled, boiled or microwaved. According to a report submitted to the Southern Supersweetcorn Council in 2001, the preparation (cooking) time was the reason that 22% of the people interviewed did not buy sweetcorn. However, sweetcorn is a convenient dish as it requires less time to be cooked in comparison to other vegetables such as carrots and broccoli. While the most common method of cooking sweetcorn is boiling, the variations in cooking methods across cities and countries are intrinsic. Microwave cooking, strengthens the advantage of ‘quick cooking’ even more as it reduces the preparation time and is not weather dependent like other methods of cooking such as outdoor grilling (Degner *et al.*, 2001). A brief literature about the effect of cooking in the attributes examined for the purposes of this thesis is provided.

Several studies comparing microwave with conventional heating, indicated that in some pathogens such as *Staphylococcus aureus*, microwave radiation was more lethal (Dreyfuss and Chipley, 1980) and that nutritional loss was equal or lower than other conventional methods (Lorenz, 1976). The blanching efficiency is usually assessed by the heat stability of peroxidase (POD) either as a soluble or insoluble peroxidase isoform (Boyes *et al.*, 1997; Barrett *et al.*, 2000). It has been found that the inactivation of POD was more efficient when samples were microwaved than water blanched even with the usual problem of non-uniform heating (Boyes *et al.*, 1997). Furthermore, it has been stated that, both lipoxygenase (LPO) and POD activity decreases with increased blanching times. In particular, it has been considered that 4 min were sufficient to inactivate LPO and 8 min for POD. The activity of these enzymes also varies between cultivars. In particular, *sh2* cultivars were found to require less time for the inactivation of LPO than *su* cultivars (Barrett *et al.*, 2000).

When vegetables are cooked, the concentration of several nutrients such as the heat-sensitive ascorbic acid and some phenolic compounds may decline depending on the type and the length of cooking (Marowa-Wilkerson *et al.*, 2007; Rickman *et al.*, 2007b). Especially for sweetcorn, it has been reported that the carotenoid content of raw frozen sweetcorn was found to be greater than for boiled frozen sweetcorn for 7 minutes (Hart and Scott, 1995). Hence, upon cooking, sweetcorn has potentially reduced health properties such as those against eye macular degeneration. On the other hand, while carotenoid content might be reduced upon cooking, it is believed that humans absorb carotenoids more effectively when consumed cooked (Marowa-Wilkerson *et al.*, 2007), but the research for this matter is still very limited.

A major problem that occurs due to cooking is the formation of brown pigments. It is believed that browning of cooked kernels is related to the Maillard reaction which occurs between amino acids and sugars during thermal processing, as sweetcorn has high levels of both compounds (Courter *et al.*, 1988), and in addition that no brown colour is developed until kernels are cooked. However, in a study by Riad *et al.* (2003), it was suggested that browning is not a result of the Maillard reaction as total phenolic content of fresh-cut kernels was not changed significantly during storage at air or CA atmosphere, while it was declined under cooking conditions. Besides, the authors did not find any 5-hydroxymethylfurfural (HMF), which is produced during the Maillard reaction. Thus, authors concluded that their results indicated that the brown colour of kernels after cooking was probably a result of phenolic-protein complex compounds. Talcott and Howard (1999), proposed the polymerization of phenolic compounds might be the reason for browning after cooking as under thermal treatments the autooxidation of polyphenolic compounds is more severe than without any heat treatment.

The browning of fresh-cut sweetcorn kernels that occurs upon cooking can be overpassed by CA storage. For instance, CA composed of 2% O<sub>2</sub> and 10% CO<sub>2</sub> proved to be successful for fresh-cut sweetcorn kernels stored at 5°C for 10 days (Riad *et al.*, 2003). The authors boiled 50 g of fresh-cut kernels in 300 ml of water for 10 min.

In the previously mentioned study, a decline in the sugar content of the fresh-cut sweetcorn kernels was also observed which was believed to be result of sugar leaking into the boiling water. Changes occurred in the sugar content, have also been examined by Barrett *et al.* (2000). Sucrose content was increased up to 4 min but did not change

thereafter. Colour properties and firmness in fresh and blanched cobs for 2, 4, 6 and 8 minutes were also examined in the same study. Hue angle and chroma was increased with increasing blanching times while lightness decreased. Hence increasing blanching time results in a lighter colour and in more intense vividness of colour. Firmness was found to be increased for up to 6 min of boiling and then declined. Thus, it can be said that firmness of cooked cobs fluctuates depends on the length of cooking time.

To summarise briefly, cooking has numerous effects on sweetcorn quality attributes such as on its nutritional value, texture and also visual characteristics regarding colour. These effects are also discussed in the relevant Chapters of this thesis (Chapter 7 and 8).

## 2.9 Conclusions

Sweetcorn is a high scale production crop which has been well studied. However several postharvest problems persist and require further investigation. Sweetcorn biology, genetics, chemical composition, texture, aroma, preharvest and postharvest factors that influence its quality and practices that can improve its quality and longevity are the most important issues that have been documented. However, the introduction of new types of sweetcorn that in the main involve sugar enhancing genes, has resulted in new varieties that can be very different even when belonging to the same genotype. This variation, in combination with the continuously increasing requirements of consumers for greater quality, feeds the need for further investigation of new hybrids with greater quality characteristics, health promoting substances and improvement of applied techniques used for the maintenance of postharvest quality.

Moreover, the increased promotion for a plant-based diet regarding the potential decrease of the development of several diseases led to the need for further research on antioxidant compounds such as antioxidant phenolic compounds, vitamins and carotenoids. Thus, a better understanding of the chemical and textural profiling of sweetcorn is required. The current work aims to provide more information on the spatial and chemical changes which occur in the most popular sweetcorn of fresh market; the supersweet sweetcorn (*sh2*-sweetcorn) during storage. In the present work, spatial

change was considered as the variation of target attributes (sugar content and/or firmness) on a vertical axis and in different tissues (kernels, core, shank) of the sweetcorn cobs. The consideration of spatial changes is further detailed in Chapter 6.

### 3 CHAPTER THREE

## **Materials, validation and optimisation of methods used for the measurement of quality parameters in sweetcorn**

### **3.1 Introduction**

Biochemical analysis of fruits and vegetables is very important for the optimisation of postharvest handling conditions by contributing to a better understanding of quality parameters. In sweetcorn, sugar content in the endosperm, which is a major quality parameter, is mainly constituted of sucrose, glucose and fructose (Zhu *et al.*, 1992). Total phenolic content of sweetcorn is also important; mainly due to potential antioxidant activity (Asami *et al.*, 2003). In terms of phenols; ferulic acid dominates among other phenolic acids which occur as products of glycosides and soluble esters. Another important component of sweetcorn is a form of vitamin C; L-ascorbic acid. Ascorbic acid is considered a very important component in foods; due to antioxidant and therapeutic properties (Okiei *et al.*, 2009).

The prevention of several degenerative diseases has been suggested to be associated with the activity of carotenoids contained in sweetcorn (Kurilich and Juvik, 1999b). Carotenoid content of kernels might also be associated with colour which is also closely related to quality in terms of appearance according to consumers (Ghorpade *et al.*, 1998). Furthermore, most of the research conducted on sweetcorn samples has been focused on carotenoid content rather than the antioxidant activity of the carotenoids. The sum of known and unknown antioxidant compounds, expressed as total antioxidant activity, is also often measured in sweetcorn cobs. Therefore, further knowledge on the total antioxidant content may be useful (Halvorsen *et al.*, 2002).

Other characteristics of sweetcorn kernels such as starch, concentration of respiratory gases inside packaging, colour of the cobs, textural characteristics and moisture content, are parameters of sweetcorn that also affect quality. In a review paper about the importance of the nutritional quality of vegetables, it is stated that there is

evidence that is better from health aspect to consume whole foods rather than their isolated components (Kader *et al.*, 2004). Therefore it shall be promoted to give further importance in consumption of sweetcorn not only for its flavour but also for its components.

The necessity of accurate methods for the analysis and measurement of quality parameters of sweetcorn, has led researchers to continue optimisation of previously described methods and the development of new ones. This chapter aims to give a brief description of the methods used from several researchers to measure the quality parameters of sweetcorn mentioned above and to mention their deficiencies in order to identify points that potentially need optimisation. The procedures used for the purposes of the experiments carried out in the current work are also described. The current chapter also attempts to validate methods already used by others and to contribute to their optimisation.

To summarise briefly the chemical measurements performed, in Experiment 1 (see Chapter 4), sweetcorn samples from seven cultivars were stored under control atmosphere conditions and kernels were analysed for their sugars content and texture (using Kramer Shear Cell). In Experiments 2 and 3, the sugar content and texture (maximum compressive load) of some other cultivars was evaluated, examining the effects of the interaction of genotype, storage time, packaging, temperature, format (with or without husks) and origin of the sweetcorn cobs on those quality parameters. Experiments 2 and 3 are described in Chapter 5. In Experiment 4 and 5 (see Chapter 6), the maximum compressive load of kernels (see section 3.6.2 –Penetration probe) and the sugar content of kernels, core and shank was evaluated, and the interaction of several factors such as format and length of shank on the characteristics examined. In Experiment 6 which is described in Chapter 7, responses of texture, sugar concentration, starch and total phenolic content to cooking were explored. The influence of cooking on textural and biochemical characteristics (sugars, total phenolics, ferulic acid and carotenoids) of supersweet sweetcorn as part of Experiment 7 are described in Chapter 8. Texture was evaluated with Kramer Shear Cell only in Experiment 1, while in all the other experiments texture was evaluated with the penetration probe. The reason for this distinction relies on the time that was available for the preparation procedure of each experiment and on the need for consistency in all samples of a specific experiment. In

particular, the method that used the Penetration probe was more time consuming than the Kramer Shear Cell method, as replicates were applied not only between samples but also within the same sample (several number of kernels- specified in each Chapter separately). Furthermore, the bulky analysis that Kramer Shear Cell applies would be inappropriate for consistent analysis of cooked vs. raw sweetcorn cobs that was required for the experiments described in Chapter 7 and 8. On the other hand, the experiments described in Chapter 6, required spatial analysis. However if the method 'Kramer Shear Cell' had been applied for that purpose, the results would be of poor validity. In more detail, the sample per section of the cobs analysed would be very weak considering that in Experiment 1, kernels had been taken from all over the cobs rather than from individual sections (bottom, middle, top). Experimental design of each experiment is described in Chapters 4, 5, 6, 7 and 8. Statistical analysis used throughout all experiments is reported in this Chapter (see Section 3.14).

### 3.2 Reagents

Reagents for the analysis of carotenoids, hexane, ethanol, methanol and dichloromethane were purchased from Fisher Scientific Chemicals (Leics., UK). Lutein, zeaxanthin,  $\beta$ -carotene,  $\beta$ -cryptoxanthin and  $\beta$ -Apo-8'-carotenal were purchased from Sigma Aldrich (Dorset, UK).

For the analysis of starch content, thermostable  $\alpha$ -amylase, amyloglucosidase, Gopod reagent buffer [Potassium phosphate buffer (0.26 M, pH 7.4), p-hydroxybenzoic acid (0.22 M) and sodium azide (0.4 % w/v)], Gopod reagent enzymes (glucose oxidase, peroxidase and 4-aminoantipyrine), D-glucose standard solution and standardised maize starch control were supplied by Megazyme Ltd (Co. Wicklow, Ireland). For the purpose of the assay, sodium acetate (FSA, Fisons specified lab. Reagent), calcium chloride dehydrate and KOH were purchased from Sigma Aldrich, and HCl, sodium hydroxide and glacial acetic acid were supplied by Fisher Scientific.

For the analysis of total antioxidant capacity, the sodium acetate trihydrate ( $\text{C}_2\text{H}_3\text{NaO}_2$ ) was purchased from Fisher Scientific, Iron III Chloride ( $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ) from Acros Organics (Leics., UK), 2,4,6-tripyridyl-2-triazine and Iron II Sulphate ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) from Sigma-Aldrich and acetic acid and HCl from Fisher Scientific.



The phosphoric acid that was used to test the identification of ascorbic acid by HPLC was purchased from Acros Organics while all the reagents used for the L-ascorbic assay used; were supplied from Megazyme. All other reagents were supplied from Sigma Aldrich unless otherwise stated.

### **3.3 Freeze-drying and moisture content**

All samples were snap-frozen with liquid nitrogen and then stored at -40°C. Approximately 20 g of the frozen kernels of all samples were weighed (Fresh Weight-FW), and then freeze-dried using a Cooling Trap, Coolsafe™ (55-9, Scanlaf A/S, Denmark) freeze-dryer and high vacuum pump (E2M5, Edwards, Sussex, UK). Dry samples were weighed again (DW) and stored briefly at -40°C until subsequent analysis. Moisture content (W) was calculated by the formula  $W = [(FW - DW)/FW] \times 100$ , where FW is the mass of kernels (g) before drying and DW the mass of kernels (g) after drying. All kernel samples were lyophilised using pestle and mortar, prior to any extraction unless otherwise stated, and core and shank samples (Chapter 6) were ground using a grinder (RMO, Retsch, Germany).

### **3.4 Respiratory gases**

#### **3.4.1 Controlled Atmosphere storage**

Controlled atmosphere (CA) storage was used to achieve 8 KPa O<sub>2</sub> and 12 KPa CO<sub>2</sub>, for the purposes of the experiment described in Chapter 4. Sweetcorn cobs were divided equally between four rigid polypropylene CA storage containers (88 cm x 59 cm x 59 cm) and removed at regular intervals. CA conditions were achieved by mixing gases [British Oxygen Company (BOC), Surrey, UK] using a gas blender (model 850, Signal Instruments, Surrey, UK). Calibration was checked by using gas chromatography (GC) as described below.

### 3.4.2 Oxygen and carbon dioxide

Oxygen and carbon dioxide in the packaging materials were determined with procedures described by Terry *et al.* (2007a). Concentrations of both gases were measured by repeated withdraws of gas into 20 mL plastic syringes and then injections of the samples into a gas chromatograph (Carlo Erba Instruments, Herts., UK) coupled to hot wire detection operated at 120°C. The oven operated at 80°C and a stainless steel column (4 mm) of 2 m length was packed with Porapak mesh of a range of 60-80 (Jones Chromatography, Mid Glamorgan, UK). The GC was calibrated using 10.06 % (v/v) CO<sub>2</sub> in N<sub>2</sub> [British Oxygen Company (BOC) gases, Surrey, UK] while O<sub>2</sub> was calibrated using ambient air.

## 3.5 Colour

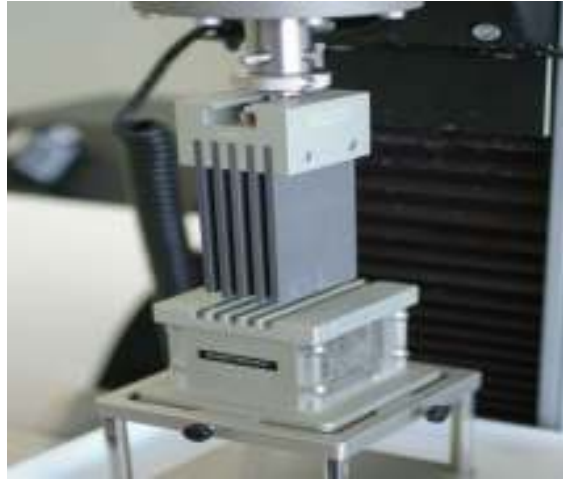
The colour of each sweetcorn sample was measured using a colorimeter (Minolta CR-400) with a DP-400 data processor (Minolta Co. Ltd., Japan). The light path of the aperture was 8 mm. A white tile (CR-400, Minolta) was used for the calibration of the instrument (*Y*: 93.5, *x*: 0.3114 and *y*: 0.3190). The instrument calculated automatically lightness (*L*\*), chroma (*C*\*) and hue angle (*h*°), upon recording the means of triplicate readings at three randomly selected points (*n*=9 per sweetcorn cob) over the equatorial axis of the surface of cobs (kernels) and when applicable of husks.

## 3.6 Texture

### 3.6.1 Kramer Shear Cell

A uniaxial testing machine (Single Column System 5542, Instron, MA., USA) equipped with Kramer Shear Cell (S5403A Series, Instron) with calibrated 500 N load cell (Figure 3.1), was used to measure the texture of kernels in the sweetcorn samples tested for the purposes of the experiment described in the Chapter 4. The Kramer Shear Cell (34.7 x 43 x 50 mm) had 5 blades of 3 mm thickness. The machine was

programmed (Bluehill 2, version 2.11, Instron) such that the crosshead speed was 10 mm/min. The maximum compressive load was recorded. Textural tests for each cob (n=210) were done on sample kernels (10 g), which were evenly distributed on the bottom within the Kramer Shear Cell.



**Figure 3.1:** Instron Testing machine-Kramer Shear Cell

### 3.6.2 Penetration probe

A penetration probe was used to evaluate firmness of the sweetcorn cobs examined (Instron, 5542, USA). In more detail, maximum compressive load was measured by using a uniaxial testing machine (Single Column System 5542, Instron, MA., USA) to determine the texture of the kernels. A photo of this device is displayed in Figure 3.2. The flat head penetration probe was of 2 mm diameter and 12 kernels of each cob were penetrated until a depth of 5 mm was reached. In the experiments where sweetcorn cobs were cooked in the microwave (Chapter 7 and 8), individual kernels were penetrated until a depth of 8 mm was reached. The machine was programmed (Bluehill 2, version 2.11, Instron) such that the crosshead speed was 20 mm/min.



**Figure 3.2:** Penetration probe

### 3.7 Sugars

Several methodologies have been reported for the analysis of sugars in fruits and vegetables and can be categorised as refractometric, chromatographic or enzymatic assays. HPLC is usually the preferable method for the identification and quantification of individual sugars in fruits and vegetables (Potus *et al.*, 1994).

#### 3.7.1 Extraction method

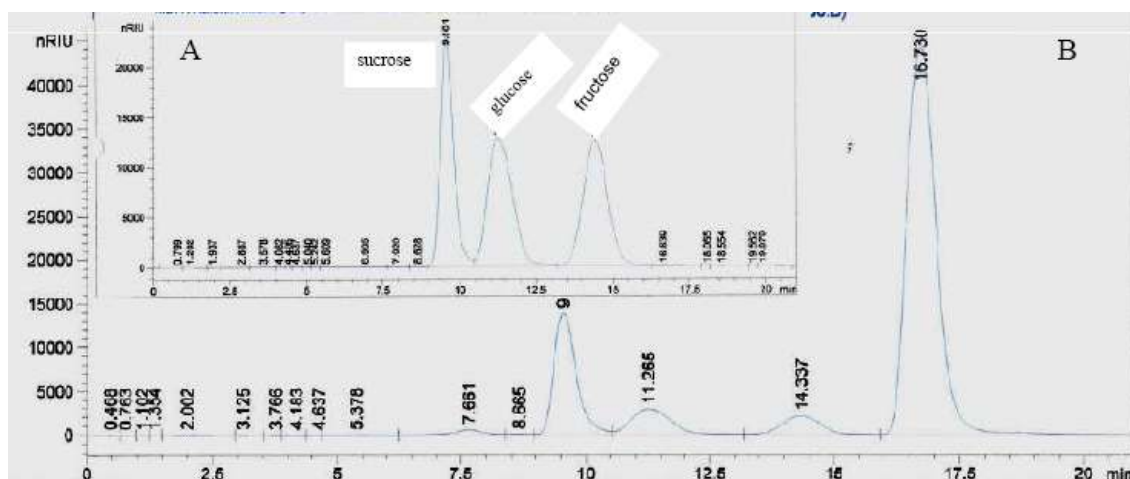
For the purposes of the current work sucrose, glucose and fructose were extracted from the kernels of sweetcorn cobs according to Terry *et al.*, (2007b). Approximately 20 g of fresh sweetcorn sample were freeze dried in an Edwards Modulyo freeze drier (W. Sussex, UK). Then freeze-dried sweetcorn kernel powder (150 mg/sample) was mixed well with 3 mL of 62.5:37.5 (v/v) HPLC grade aqueous methanol solution and vortexed (Vortex Genie 2, Scientific Industries, NY) to mix thoroughly. The extracts that were contained in 7 mL polystyrene bijoux vials (Sterilin, Staffs., UK) and were then placed in a shaking water bath at 55°C for 15 min. The vials were removed from the water bath every 5 min and vortexed in order to avoid formation of layers. Sugar extracts were then filtered through 0.2 µm syringe filters (Millipore Corp., MA, USA) and diluted with HPLC grade water (1:10) prior to analysis.

### 3.7.2 Quantification of sugars

#### 3.7.2.1 Quantification of identified sugars

Sugar extracts (20  $\mu$ L) were injected into a Rezex RCM monosaccharide  $\text{Ca}^+$  (8%) column of 300 x 7.8 mm diameter and particle size 8  $\mu$ m (00H-0130-K0, Phenomenex, CA.), with a carbo  $\text{Ca}^{2+}$  guard column of 4 x 3mm diameter (AJO-4493, Phenomenex) and quantified using an HPLC system (1200 series, Agilent Technologies, Berks., UK). The mobile phase was HPLC grade water at a flow rate of 0.6 mL/min and the temperature of the column was held at 80°C using a temperature-controlled column compartment (G1316A, Agilent). Eluted carbohydrates were monitored by a refractive index detector (RID, G1362A, Agilent) connected to the HPLC system, which included a cooled autosampler (G1330B, Agilent) set at 4°C. The presence and quantity of sucrose, glucose and fructose were calculated by comparison of sample peak area to calibration standards (Sigma, Dorset, UK), ranging in concentration from 0.025 to 2.5 mg/mL, using ChemStation software (Rev. B.02.01, Agilent). Retention times were 9.801, 11.644 and 14.434 min for sucrose, glucose and fructose, respectively (Figure 3.3). Limits of detection were 42.84, 18.48 and 5.55 mg/mL for sucrose, glucose and fructose respectively. Sweetness was calculated by using the following coefficients: fructose (1.2), sucrose (1) and glucose (0.64) (Kader, 2008).

In some chromatograms, such as in Figure 3.3, other peaks apart from the three known were apparent, indicating the presence of other sugars apart from sucrose, glucose and fructose. Considering previous works that had found maltose (Ferguson *et al.*, 1979) and raffinose (Baird *et al.*, 1996) in sweetcorn kernels, d-maltose-monohydrate (Sigma Aldrich, UK) and d-raffinose-pentahydrate standards were run to check any possible matches. Results showed that neither maltose nor raffinose, were identified in the samples tested.



**Figure 3.3:** Representative chromatogram of eluted sucrose, glucose and fructose in standards (A) and in sweetcorn extracts (B).

### 3.7.2.2 Alternative method for quantification of sugars in sweetcorn

Extracts (n=20) prepared from samples examined for the purposes of the experiment described in Chapter 4, were also analysed as described by Chope *et al.* (2007). In particular, an HPLC system with a P580 pump and Gina 50 autosampler (Dionex, CA) were used; in order to compare the results with the device that was finally used. This comparison would allow a validation of the results and in addition would contribute to a final conclusion of whether ELSD (evaporative light scattering detector) or RID was more appropriate for the detection and quantification of sucrose, glucose and fructose. To detect the eluted carbohydrates in this case; an ELSD (2420, Waters, MA, USA) was used. An UCI-50 universal chromatography interface was used to connect the detector to the system. The column temperature was set at 75 °C using a Dionex STH column thermostat. All the other conditions were identical to the method used and described in section 3.7.2.1. The presence and quantity of the sugars of interest were calculated as described before using Chromeleon, version 4.6 software. The comparison between the two different methods of HPLC analysis of individual sugars indicated that the results obtained when the refractive index detector was used, did not differ significantly from the results obtained when the light scattering detector was used indicating that both detectors are equally suitable for sugar analysis for sweetcorn.

### 3.8 Total soluble solids

Among several analytical techniques targeting sugars, such as colorimetric and chromatographic techniques which are either expensive and/or time-consuming, refractometric techniques are occasionally the preferred methods for sweetness determination. Refractometric techniques offer simple and fast determination of sugar content in °Brix, without preparation steps but are not always well correlated with sugar content (Gine Bordonaba, 2010). For the purpose of the experiment described in Chapter 8, total soluble solids (TSS) content in the water remaining in the microwavable bowl after cooking, was measured by a digital refractometer (PR 301 $\alpha$ , Atago Ltd., Japan).

### 3.9 Total starch

Starch components (amylase and amylopectine) can generally be measured by Near Infrared Spectroscopy, enzymatic methods and high-performance size-exclusion chromatography, involving polarimetric and acid hydrolysis or enzymic procedures. However, these methods are sometimes problematic, as they can be very complicated, expensive or time-consuming (McCleary *et al.*, 1994; Stawski, 2008). Starch gelatinization, hydrolysis of dextrans to glucose, glucose determination, starch liquefaction and dextrinisation and pre-treatment steps are varied in enzymic procedures (Knudsen, 1997). Nowadays, treatment with thermostable  $\alpha$ -amylase during or after starch gelatinisation is often used (McCleary *et al.*, 1994).

Based on the conclusions of these works, Megazyme produced a total starch assay kit which incorporated  $\alpha$ -amylases which are active and stable at even lower pH conditions than in the past. In particular, the advantage of this method which is commonly known as amyloglucosidase/  $\alpha$ -amylase method; is that due to incubation steps of thermostable  $\alpha$ -amylases and amyloglucosidases at the same pH (5), the production of maltulose, which is not easily hydrolysed by amyloglucosidase and  $\alpha$ -amylase, is minimised (McCleary *et al.*, 2009). The method suggested by Megazyme, was later adopted by the Association of Analytical Communities (Method 996.11) and

the American Association of Clinical Chemistry (Method 76.13). The same method was also used to determine the total starch content in sweetcorn samples, for the purposes of the current work.

The principle is based on production of maltodextrins after hydrolysis of starch due to presence of thermostable  $\alpha$ -amylase, at pH 5 and 100 °C. Maltodextrins are then hydrolysed quantitatively to D-glucose due to the presence of amyloglucosidase (AMG). D-glucose is then oxidised to D-gluconate. In that reaction, each mole of hydrogen peroxide produced, results in a colour change which is then measured using a spectrophotometer. The detection limit of this assay is 1.8 mg of starch/L or 2.0 mg of D-glucose.

The extraction method used for the determination of starch in the sweetcorn cobs was carried out as follows. Samples of ground freeze-dried sweetcorn powder (100 mg) were incubated with 5 mL of 80% (v/v) aqueous ethanol at 80 °C for 5 min. After vortexing the solution (Vortex Genie 2, Scientific Industries, NY); another 5 mL of 80% (v/v) aqueous ethanol was added. Samples were then centrifuged for 10 min at 1,800 g. The supernatants were discarded and the pellets were re-suspended in 10 mL of 80% (v/v) aqueous ethanol and mixed again in a vortex stirrer. Samples were then centrifuged as before and the supernatant once more discarded. Immediately, 3 mL of thermostable  $\alpha$ -amylase diluted 1:30 in 100 mM sodium acetate buffer (pH 5), was added. Incubation of the samples for 6 min in a boiling water bath followed. Samples were stirred after 2, 4 and 6 min in order to ensure homogeneity of the slurry. After the addition of 0.1 mL of amyloglucosidase, contents of the tubes were mixed and incubated at 50°C for 30 min. The contents of the tubes were then adjusted to 100 mL with distilled water and mixed thoroughly. Aliquots of these solutions were centrifuged at 1,800 g for 10 min. Duplicate filtered aliquots (0.1 mL) were then transferred to glass tubes; and after the addition of 3 mL of Glucose Determination Reagent (Gopod Reagent: Glucose-oxidase-peroxidase Reagent), incubation at 50°C for 20 min followed. In more detail, Gopod Reagent used as supplied by Megazyme, consisted of peroxidase (>650 U); glucose oxidase (>12000 U); 80 mg 4-aminoantipyrene and 1M potassium phosphate buffer mixed with 0.22M p-hydroxybenzoic acid and 0.02% (w/w) diluted with distilled water. D-glucose control samples consisted of 1 mg/mL D-glucose standard solutions and 3 mL of Gopod Reagent. The absorbance of each sample and D-



glucose control were read at 510 nm against reagent blank. Results were expressed as g/100 g (%) of kernels on dry weight basis. Generally avoidance of interference in this assay can be validated by completion of D-glucose at approximately 5 min which is the time specified by the assay. Thus, D-glucose was added (500 µg/mL) and indeed a significant increase in the absorbance was observed.

The method used to examine the starch content, was proved to be a valid method for the analysis of starch content in sweetcorn samples.

### **3.10 Organic acids and L-ascorbic acid**

#### **3.10.1. Methods tested for identification of organic acids and L-ascorbic acid**

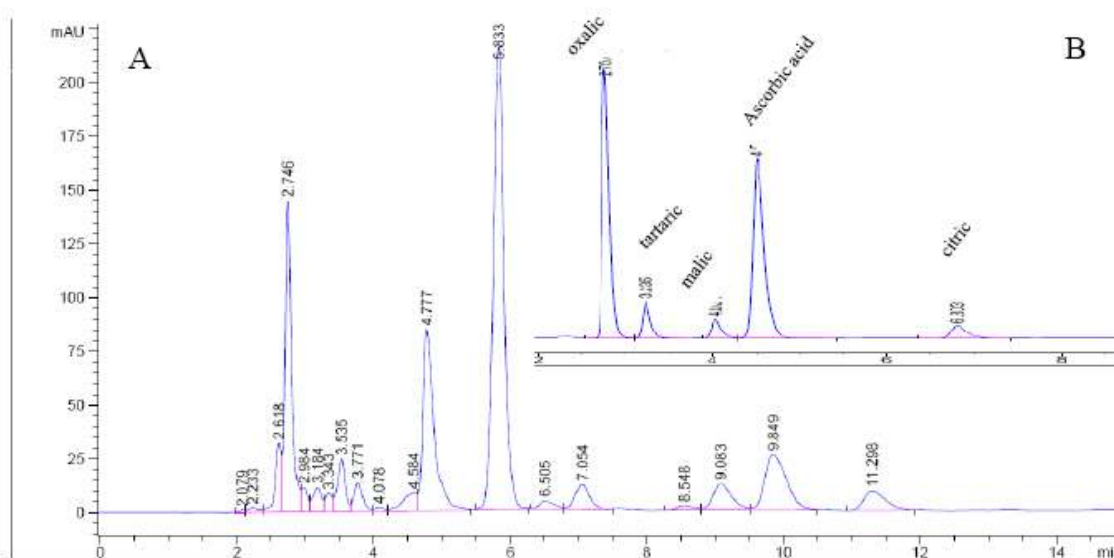
In addition to sugars, organic acids are very important components in sweetcorn which also have the ability to influence pH and the production of off-flavours in fruits and vegetables (Zagory and Kader, 1989). The target of the current work was the analysis of L-ascorbic acid which is a water soluble antioxidant; plentiful in plants. Titration and spectrophotometry are two frequently used methods for the analysis of organic acids, including vitamin C (Salkic *et al.*, 2007). Amperometry and chromatography can also be used (Korany *et al.*, 2010). However, it should also be noted that analytical methods for organic acid analysis, are not always reliable due to effect of compounds being oxidised (Arya *et al.*, 2000).

For the determination of ascorbic acid, a method for the extraction of non-volatile organic acids in strawberries described by Terry *et al.* (2007b); was examined as a possible method for the analysis of vitamin C in sweetcorn samples. In more detail, different quantities of lyophilised sweetcorn samples (100, 150, 200, 150, 300 mg), were used and compared for the preparation of ascorbic acid extracts. Each sample was then mixed with 3 mL of HPLC grade water and kept at room temperature (25°C) for 10 min. After, extracts (n=3) were filtered through 0.2 µm syringe filters ready for analysis.

It was attempted to determine L-ascorbic acid by an HPLC system as described in sugars section, equipped with an Agilent DAD G1315B/G1365B photodiode array with multiple wavelength detector. Sweetcorn extracts (20 µl) were injected into an Alltech Prevail Organic acid column of 150 mm x 4.6 mm diameter. Particle size of the

column was 5  $\mu\text{m}$  (Alltech, CA; Part no. 88645) and the guard column used was an Alltech Prevail Organic Acid guard column of 7.5 mm x 4.6 mm diameter (Alltech, CA; Part no. 96429). Aqueous grade and degassed metaphosphoric acid (Fisher Scientific) 0.2% (w/v) was used as a mobile phase with 1 mL/min flow and under isocratic conditions. The mobile phase was adjusted to pH 2.5 using phosphoric acid. The temperature of the column and the autosampler was set up at 35 and 4°C, respectively. The eluted organic acids were detected at 210 nm and the presence of organic acids identified by comparing their peak areas with standards, using ChemStation rev. B.02.01 software.

A representative chromatogram from the analysis of organic acids using 100 mg of sweetcorn kernels is shown in Figure 3.4. Peaks were better separated when 100 mg of sample was extracted for organic acid quantification, rather than from greater amounts (see Appendix A). Known concentrations of succinic, malic, citric and  $\alpha$ -ketoglutaric acid reported by Masuda *et al.* (1997) were also analysed in an effort to identify other organic acids from the sweetcorn kernels, but results indicated that none of the pure compounds analysed were present in the samples tested. Therefore, this procedure was not chosen as the preferred analytical method for L-ascorbic acid in the sweetcorn cobs examined. The method that was finally assayed for the analysis of L-ascorbic acid is described in the next subchapter (see section 3.10.2).



**Figure 3.4:** Representative chromatogram obtained from the analysis of (A) organic acid standards and organic acids (B) from sweetcorn extracts.

### 3.10.2 L-ascorbic acid assay: principles of extraction and optimisation of the analysis method

Samples were tested for the identification and quantification of L-ascorbic acid by using the L-ascorbic acid assay kit bought from Megazyme, with some modifications. The principle of the assay is that a formazan compound is formed when 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is reduced in the presence of reducing substances such as L-ascorbic acid and the electron carrier PMS (phenazinemethanesulphate) at pH 3.5. The difference in absorbance at 578 nm, between sample and sample blank (sample that other reducing substances have been measured when L-ascorbic acid has been removed using ascorbic acid oxidase) indicates the quantity of the L-ascorbic acid.

To prepare the samples, 20 g of sweetcorn were mixed well with 50 mL of 100 mM potassium phosphate buffer (pH 3.5). Samples were also tested for their L-ascorbic acid concentration starting with different amounts of fresh sample (25 and 30 g), different volumes of potassium phosphate buffer and different molarities of the buffer (1 M), but in each case, results were inferior or not significantly different compared with the method used (see Appendix A).

The pH of the slurry was adjusted to 3.5 with 2 M HCl. The mixture was then adjusted to 300 mL with distilled water and after re-adjusting to pH 3.5, was mixed and filtered. Briefly, 1 mL of each sample, with 0.62 mL of distilled water (warmed to 37°C) and 0.5 mL of phosphate/citrate buffer and a sample blank for each sample were mixed and incubated for 3 min at 37°C. When less than 1 mL of sample was used in the combination above, concentration of L-ascorbic acid was below the detection limit of the assay. The sample blank also had the same final volume of 2.52 mL with the addition of 0.02 mL ascorbic acid oxidase. After incubation, samples were incubated again for 3 min in the same conditions with the addition of MTT/acetate buffer and the absorbencies of both samples and sample blanks were read. The addition of 0.2 mL PMS solution in a light-protected environment at 37°C, initiated the reaction. After 8 min, absorbance was measured again. The timing of the last measurement was determined after observing no significant difference in the concentration of L-ascorbic acid thereafter. Furthermore, standard addition at the end of the reactions resulted in a

significant increase in the absorbance showing that the reaction was completed. The detection limit of the assay was 0.0175 mg/L. Finally, the method was tested with the same material but using freeze-dried sweetcorn powder as the initial sample. In this case, concentrations of L-ascorbic acid were not only found to be significantly lower when dried samples were used; but readings were also below detection limits. Results from the comparison of initial fresh versus dried samples are also shown in Appendix A.

To test the end of the reaction, sweetcorn samples were spiked with a known amount of L-ascorbic acid standard. The reproducibility of the procedure was investigated between three separate extractions ( $n=9$ ) of a tissue sample of a randomly chosen sweetcorn cob and also between three aliquots obtained from the same extraction. The variability within samples and sample replicates and the variability within extracts were estimated by using means and standard deviations. In particular, results as shown in the Table 3.1 below indicate that reproducibility of the method is high due to the low variation of the extraction procedure and the sample replicates.

**Table 3.1:** Reproducibility results: L-ascorbic acid in aliquots of the same extract and different extracts of the same sample tissue.

<b>Cooking duration</b>	<b>Number</b>	<b>Same extract</b>	<b>Different extract – Same tissue sample</b>
0min (fresh)	3	$3.51 \pm 0.33$	$3.82 \pm 0.40$
Cooked for 5min	3	$2.40 \pm 0.27$	$2.35 \pm 0.30$
Cooked for 10min	3	$2.72 \pm 0.22$	$2.44 \pm 0.50$

\* Standard deviation and mean concentrations are expressed as mg/100g of L-ascorbic acid in fresh sweetcorn kernels.

The method used to quantify L-ascorbic acid concentrations in the sweetcorn samples using HPLC as previously described by other researchers was not successful. However, the existence of other well separated, unidentified organic acids was demonstrated. The Megazyme assay kit for the measurement of L-ascorbic acid was suitable for sweetcorn samples. The method was specified and optimised for sweetcorn kernels. Asami *et al.* (2003) suggested that frozen corn samples were suitable for HPLC

analysis of L-ascorbic acid, while freeze-dried initial samples were not appropriate. The method used for the current work reconfirmed this conclusion.

### 3.11 Phenolic compounds

#### 3.11.1 Total phenolics

Several methods have been developed to determine phenolic compounds in fruits and vegetables, with HPLC and spectrophotometric methods having several advantages and disadvantages. In more detail, HPLC analysis of phenolic compounds, while accurate, is complicated, time-consuming, has high cost and like any HPLC assay relies on available standards in identifying individual compounds. On the other hand, spectrophotometric methods are simple, less time-consuming and when are performed under consistent conditions can offer information on total phenolic content. In the current study determination of phenolic compounds was attempted by HPLC analysis (see section 3.11.3), but as only ferulic acid was identified; spectrophotometric measurement of total phenolics by the Folin-Ciocalteu assay was employed (Riad, 2004; Stratil *et al.*, 2006).

For the extraction and quantification of total phenolics, 150 mg of freeze-dried ground powder per sample of sweetcorn kernels was used (Terry *et al.*, 2007b). The samples were dissolved with 3 mL of 80% aqueous ethanol (v/v) and after stirring in a vortex device were placed in a water bath (HAAKE SWB 20, Thermo Scientific, Germany) set at 70°C, for 2 h. Mixing of the samples took place every 20 min during the 2 h incubation. After solutions were filtered samples were ready for analysis.

The Folin-Ciocalteu method according to Singleton and Rossi (1965) was used to measure total phenolics. The method is based on the ability of phenolic compounds to cause reduction of a phosphowolframate- phosphomolibdate complex forming molybdenum-tungsten blue reaction products. However, according to Stratil *et al.*, (2006) saccharides and ascorbic acid may interfere with this method. In detail, for the analysis of total phenolics, 20 µL of each filtrate was combined with 3.2 mL of distilled water and 200 µL of Folin-Ciocalteu reagent. Solutions were then mixed with 600 µL of 1.9M sodium carbonate. Final solutions were then incubated at room temperature in the

dark for 2 h. A Camspec M501 (Camspec Ltd., Cambs, UK) UV/vis spectrophotometer, was used to measure the absorbance at a wavelength of 760 nm. Measurements were calibrated against gallic acid standards (25, 50, 100, 150, 250 and 500 mg/L) and expressed as mg of gallic acid equivalents (GAE) per gram of kernels.

The method used for the analysis of total phenolics might not be very accurate as it is susceptible to interference from sugars, however it can be considered adequate for the purpose of this study which mainly focused on the comparison of fresh versus cooked cobs.

### **3.11.2 Extraction of ferulic acid**

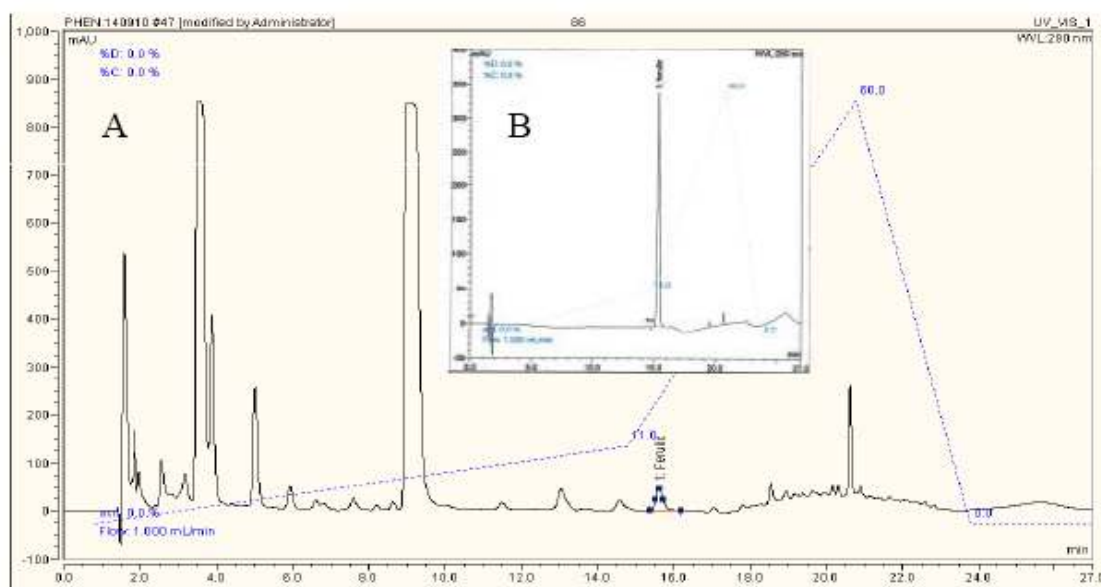
Sweetcorn samples were extracted for the analysis of ferulic acid according to the acid hydrolysis method described by Ancos *et al.* (2000) with minor modifications. In particular, 300 mg of lyophilised sweetcorn sample was mixed with 5 mL of 60% (v/v) MeOH that contained 125 µg butylated hydroxytoluene (BHT). Then, 1 mL of 6 M HCl was added to the extract. Extracts were then placed in a water-bath at 90°C for 2h and were mixed every 30 min. Undiluted filtered extracts (filtration through 0.2 mm filters) were ready for analysis.

### **3.11.3 Quantification of ferulic acid**

Extracts (10 µL) were injected into an Agilent Zorbax Eclipse Column (XDB-C18, 4.6 mm x 150 mm of 5 µm particle size) coupled with a 1.0 mm x 17mm guard column (Agilent Zorbax Eclipse XDB). The mobile phase used consisted of two filtered and degassed solvents; where solvent A was 8% acetic acid in 2 mM sodium acetate and solvent B was 100% acetonitrile. The oven temperature was set at 30°C and the UV detector at 280 nm. The flow rate was constantly set at 1 mL/min. The gradient involved % solvent B as follows: 0-11%, 14 min; 14-60%, 23 min and the post-run had 4 min duration at 0% of solvent B. Ferulic acid was calculated with linear regression analysis using external standards and the results were expressed as µg of ferulic acid per g of dry and fresh sweetcorn kernels.

Known concentrations of phenolic compounds were analysed to compare retention times and spectra with the peaks of the chromatograms. The compounds chosen for that purpose were flavonols (quercetin, myricetin and kaempferol) and phenolic acids (ferulic acid, vanillic, *p*-coumaric and protocatechuic). These compounds were chosen according to previously reported biochemical profiling on sweetcorn (Dewanto *et al.*, 2002; Trombino *et al.*, 2004 and Pedreschi and Cisneros- Zevallos, 2006). Except for the ferulic acid, analyses could not prove the existence of the compounds mentioned above in sweetcorn. There was no further effort to find alternative method of analysis that would allow the identification and quantification of these compounds as their concentrations were expected to be insignificant in comparison with ferulic acid.

The method used for the analysis of ferulic acid, revealed other compounds that were unidentified (Figure 3.5). However, identification and quantification of ferulic acid (Donetti, unpublished) was adequate for the aims of this work as it is the most abundant ferulic acid found in corn (Dewanto *et al.*, 2002).



**Figure 3.5:** Chromatogram of ferulic acid from sweetcorn extracts (A) and ferulic acid standard (B).

## 3.12 Carotenoids

### 3.12.1 Extraction of carotenoids

From the carotenoids found in sweetcorn, lutein and zeaxanthin are of particular interest due to their association with eye health (Ribaya-Mercado and Blumberg, 2004). Hart and Scott (1995) published a paper, reporting inaccuracies and variation occurring in the quantification of carotenoids as a result of several factors including light, heat and physicochemical reactions such as degradation. They also reported that the use of temperature controlled system, appropriate column and solvent are essential for ensuring the successful isolation and analysis of carotenoids. Solvent modifiers are very important when added to the mobile phase. In particular, they reported that addition of triethylamine (TEA) in the mobile phase can improve recovery of carotenoids from the column and reduce retention times.

Recently, Fanning *et al.* (2010) suggested that saponification and heating in the samples they examined had no effect on carotenoid content. However, earlier work by Howe and Tanumihardjo (2006), which compared several methods for the extraction of carotenoids, suggested that for the extraction of carotenoids from corn samples, heating and saponification was necessary for reliable results and good extraction efficiencies. Considering that Fanning *et al.*, did not report unfavourable effects of heating and saponification while other researchers reported beneficial effects, these steps were not avoided in the current work. Thus, and also considering that the purpose in this study was to evaluate the interaction of cooking time, format, storage duration and temperature on carotenoid content, a combination of methods was used as described below.

A combination of the extraction methods described by Kurilich and Juvik, (1999b); Fanning *et al.*, (2010); and Burt *et al.*, (2010), was used for the aims of the experiment described in Chapter 8. Briefly, solubilisation of the sample with ethanol and saponification with KOH under heat was the basis of the extraction procedure. The addition of KOH, was performed in order to hydrolise potential esterified xanthophylls (Schlatterer *et al.*, 2006). The extraction was then performed with hexane.



The extraction procedure used was as follows: 500 mg of freeze-dried samples were mixed with 6 mL of analytical grade ethanol containing 0.1 % butylated hydroxytoluene (BHT) and 800  $\mu$ L of  $\beta$ -Apo-8'-carotenal (7.2 mg/L in isopropanol). Instead of 800  $\mu$ L of  $\beta$ -Apo-8'-carotenal, 250  $\mu$ L of this internal standard was tested as described by Fanning (2010). However, resolution of the peaks was better when 800  $\mu$ L was used. Extracts were then placed in a water bath set up at 85°C for 5 min. Potassium hydroxide (180  $\mu$ l) was then added, vortexed for 20 sec and then samples were placed back into the water bath for 10 min. During the 10 min of saponification, samples were vortexed one more time and after the end of this procedure, samples were placed immediately in an ice bath. The addition of 3 mL of hexane followed after the addition of 3 mL of distilled water. Samples were then extracted three times with 3 mL of hexane. A centrifuge (Heraeus, labofuge, 400R, Thermo Scientific) was used to separate layers (1200 g for 10 min, at 4°C) and the upper layer removed and placed in a new test tube. The hexane fractions were then combined and dried in a rotary evaporator (Buchi Rotovapor, Buchi Labortechnik AG, Flawil, Switzerland) under vacuum at 30°C. Reconstitution of samples occurred using 2 mL of methanol/dichloromethane mixture (50:50 v/v) containing 0.1 % BHT. Alternatively, 5 samples were reconstituted with 2 mL of the same solvent as used for the mobile phase A, yet no significant difference was observed. The extraction procedure was performed under fluorescent light. Filtered samples were then analysed immediately.

### 3.12.2 Analysis and qualification of carotenoids

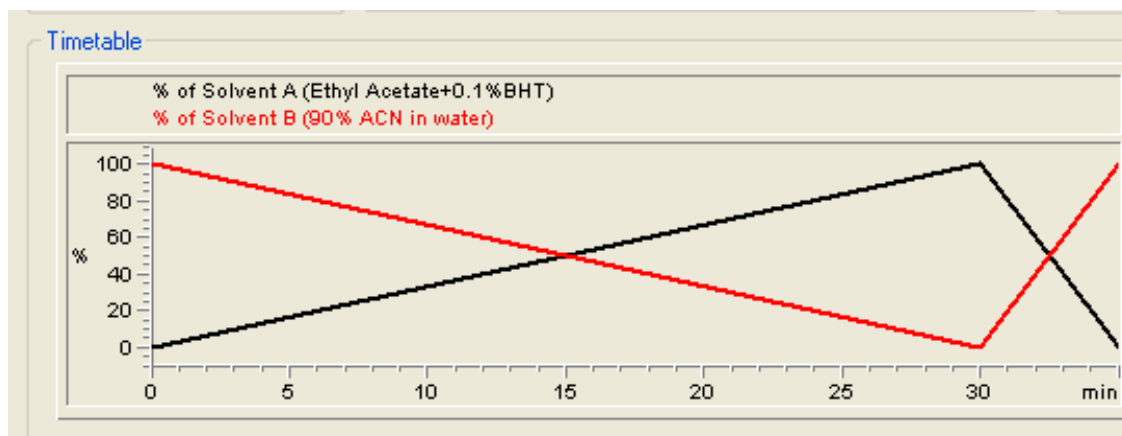
Several HPLC methods have been used for the analysis of carotenoids in sweetcorn (Weber, 1987; Kurilich and Juvik, 1999a). In the present work, carotenoids were determined by an HPLC system, equipped with an Agilent DAD photodiode array with a multiple wavelength detector (G1315B/G1365B). Sweetcorn extracts (20  $\mu$ l) were injected into a Zorbax Eclipse Column Agilent Zorbax Eclipse Column (XDB-C18, 4.6 mm x 150 mm of 5 m particle size) and the guard column used was an Agilent Zorbax Exlipse XDB coupled, of a 1.0 mm x 17 mm diameter (Kalogirou, unpublished). Alternatively, the same column of different length (XDB-C18, 4.6 mm x 250 mm of 5

m particle size) was used but no significant changes in the concentration of carotenoids were observed.

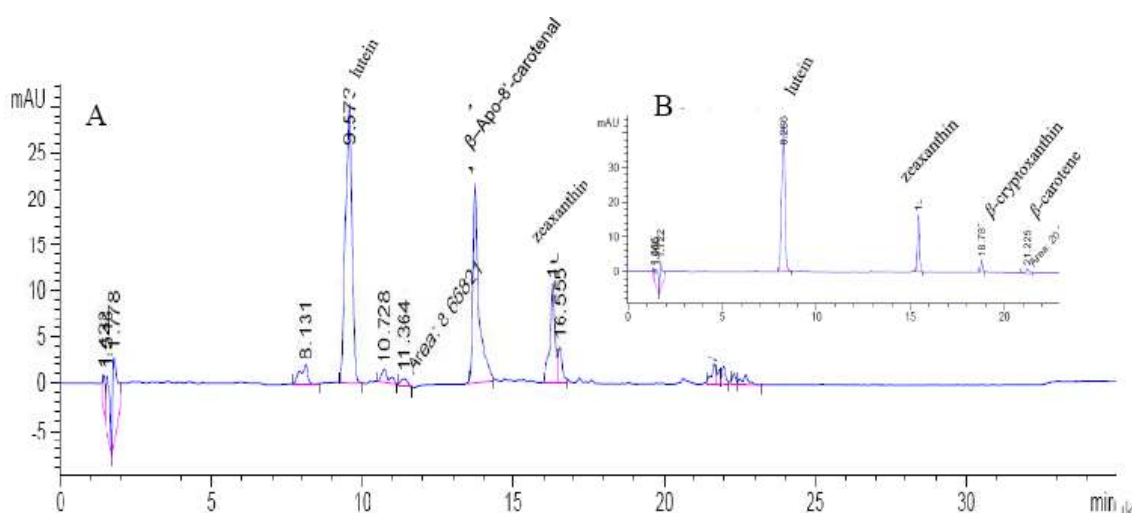
The binary mobile phase that was used at a flow rate of 1 mL/min, consisted of ethyl acetate containing 0.01% (w/v) BHT and 0.1% (v/v) triethylamine (phase A), while phase B consisted of 90% ACN in water. The temperature of the column was set at 30°C and the autosampler at 4°C. The eluted carotenoids organic acids detected at *ca.* 453 nm (Kopsell *et al.*, 2009). Retention times and absorption spectra, viewed using ChemStation rev. B.02.01 software, were used to identify lutein, zeaxanthin,  $\beta$ -carotene and  $\beta$ -cryptoxanthin. Concentrations of carotenoids were expressed as  $\mu\text{g/g}$  on fresh and dry weight basis.

Carotenoids were identified with spike tests and against retention times and absorption spectra of known standards. Lutein standards were made up in absolute ethanol and its absorbance measured using a M501 UV/Vis spectrophotometer (Camspec Ltd., Cambs., UK) at 445 nm, while zeaxanthin,  $\beta$ -carotene and  $\beta$ -cryptoxanthin were made up in hexane and measured at 450 nm (Wrolstad *et al.*, 2004). The concentration of the standard solutions was measured according to the same author, using the following formula:  $\text{Concentration} = (\text{absorbance} \times 10000)/A_{1\%}$ , where  $A_{1\%}$  (the spectral absorption coefficient) is 2550, 2480, 2592 and 2460, for lutein, zeaxanthin,  $\beta$ -carotene and  $\beta$ -cryptoxanthin, respectively. The peak purities were then determined by HPLC analysis. The concentration that was determined spectrophotometrically multiplied by the % peak area as measured by HPLC, was the actual concentration of the standard solutions. Standards were prepared at five concentrations, dried down under a stream of nitrogen gas and then reconstituted as samples. The range of the concentrations was 0.016-150  $\mu\text{g/mL}$ .

The gradient used was as shown in Figure 3.6.



**Figure 3.6:** Gradient of solvents A and B as used for the extraction of carotenoids in sweetcorn



**Figure 3.7:** Representative chromatogram obtained from the analyses of carotenoids (A) in samples and (B) in standard solutions.

Concentration of samples was calculated by comparison of their peak areas and the peak areas of the standards. Representative chromatograms of both samples and standards are shown in Figure 3.7. Coefficients of determination ( $R^2 > 99\%$ ) obtained from the known standards were used to determine the actual concentrations of the samples tested.

**Table 3.2:** Reproducibility of results regarding aliquots from the same extraction (n=3) and among different extracts of the same sample (n=3), expressed as  $\mu\text{g/g}$  on dry weight basis.

Carotenoids	Number	Same extract	Different extract – Same tissue sample
Lutein	3	$19.7696 \pm 0.1568$	$19.1201 \pm 0.4320$
Zeaxanthin	3	$0.2327 \pm 0.0090$	$0.2139 \pm 0.021$

\* Standard deviation and mean concentrations are expressed as mg of carotenoids per Kg of fresh sweetcorn kernels.

Three randomly chosen samples, were used to test the accuracy and the reproducibility of the method used between extractions of the same sample. Furthermore, three aliquots of the same extract were analysed to estimate the variability within extracts. Reproducibility concerning the same factors was estimated by means and standard deviations.

The reproducibility of the extraction procedure was considered high, since, as shown in the Table 3.2, the variation was low. Recovery values (%) were  $95 \pm 9$  for lutein and  $93 \pm 12$  for zeaxanthin indicating good accuracy of this method. The recovery value for  $\beta$ -cryptoxanthin is greater than 100% but has a standard deviation of 11%. These arithmetical data might mean that in case that recovery value is greater than 100% there is a possibility of interference of other substances. However, due to the comparison of the absorption spectra of  $\beta$ -cryptoxanthin of the sample with this of the standards, this case is eliminated. Thus, practically it can be said that the recovery value for  $\beta$ -cryptoxanthin was in a range of 99-100%. Limits of detection were 3.2602, 0.0400, 0.0286, 0.0036 and limits of quantification 10.8674, 0.0954, 0.1336 and 0.012  $\mu\text{g/g}$ ; for lutein, zeaxanthin,  $\beta$ -cryptoxanthin and  $\beta$ -carotene respectively. However,  $\beta$ -cryptoxanthin was not present in the samples and examined peaks of  $\beta$ -carotene were not always well separated and occasionally below limits of quantification and therefore were not included in the calculation of total carotenoids.

The extraction and analysis of carotenoids proved to be an effective and accurate method for the analysis of the xanthophylls lutein and zeaxanthin for the sweetcorn cobs

tested. Better chromatograms were achieved when 800  $\mu\text{L}$  of internal standard was used as Fanning *et al.* (2010) suggested; compared to 250  $\mu\text{L}$  that Howe and Tanumihardjo, (2006) used. The use of the longer C-18 Zorbax Eclipse Column (250 mm) and the reconstitution of the dried extracts with the solvent used for phase A of the HPLC analysis did not affect the results and therefore could be used alternatively. The method used for the analysis of carotenoids can be considered to be of good accuracy and reproducibility.

### 3.13 Total antioxidant activity

The FRAP (ferrous reducing antioxidant power) assay as described by Benzie and Strain (1996) and as modified by Terry *et al.* (2007a), was used to measure total antioxidant capacity. The assay is based on the reduction of a ferric-tripyridyltriazine complex, in the presence of antioxidants and measures electron-donating antioxidants. An important reason, that it was FRAP that was chosen to assay the antioxidant activity of the sweetcorn samples examined, is that is a direct method for measuring antioxidants using reductants as antioxidants without pretreatment steps. The use of antioxidants as reductants can also be the disadvantage of the FRAP assay. On the other hand in indirect methods as listed in subchapter 2.4.6, the reactive species used may significantly affect the results as they might measure antioxidants that react against the radicals used (Halvorsen *et al.*, 2002; Nagah and Seal, 2005).

For extraction, 150 mg of freeze-dried powder was mixed with methanol:  $\text{H}_2\text{O}$ :  $\text{HCl}$  (70:29.5:0.5%, v/v) solution placed in a waterbath (HAAKE SWB 20, Thermo Scientific, Germany) at  $35^\circ\text{C}$  for 1.5 h. Samples were agitated every 15 min. Before analysis, samples were filtered through 0.2  $\mu\text{m}$  Millex- GV syringe driven filters (Millipore Corporation, MA, USA). For the analysis of Total Antioxidant Capacity, 50  $\mu\text{L}$  aliquots of undiluted sample extract were mixed with 3.6 mL of FRAP working solution which was freshly prepared each day. The FRAP working solution consisted of 5 mL of 10 mM 2,4,6-tripyridyl-2-triazine in 40 mM  $\text{HCl}$  and 5 mL of 10 mM  $\text{FeCl}_3$  in 50 mL of 100 mM acetate buffer. After the addition of the FRAP solution, the mixture was incubated at  $37^\circ\text{C}$  for 10 min. The absorbance was then measured using a spectrophotometer (in a Camspec M501 UV/vis) at 593 nm. Measurements were read

against  $\text{Fe}^{2+}$  ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) standards of 0, 0.2, 0.4, 0.8, 1, 1.2, 1.6 and 2 mM concentration and expressed as the concentration of antioxidants that have ferric reducing ability.

FRAP assay has constant and linear stoichiometric factors (Halvorsen *et al.*, 2002) and when performed was proved to be simple and rapid. Therefore FRAP assay can be a good, direct method for the measurement of antioxidant activity.

### 3.14 Statistical analysis

All statistical analyses were carried out using Genstat (Version 10.1, VSN International Ltd., Herts., UK). Least significant differences (l.s.d.) between factors-sources of variation and their interaction were analysed through analysis of variance (ANOVA). Complex experimental analyses such as those required for the experiments described in Chapter 6 and 8, were adjusted, under advice from Pat Bellamy [Head of Statistics at Cranfield University] to have a maximum 3-way interactions. The analysis of variance was helpful in providing information about the effect and interaction of temperature, cultivar, type of tissue, position in tissue, format of the cobs, cooking and storage duration. All statistical analyses are reported in Appendix B. Unless otherwise stated significant differences were  $P < 0.05$ . Standard errors are presented, where l.s.d. was not appropriate.

## 4. CHAPTER FOUR

### **Biochemical and textural profiling of seven UK-grown *sh2* sweetcorn cultivars (*Zea mays L.*) held under controlled atmosphere conditions**

#### **4.1 Introduction**

Sweetcorn is an important crop in the fresh vegetable market. As consumption increases, greater knowledge is required on quality parameters (*viz.* texture, concentration of sugars) which are intimately associated to consumer acceptability. Deterioration of the quality of sweetcorn after harvest is rapid even for *sh2*-types (supersweet) which tend to reduce the conversion of sugars to starch. Increasingly, consumers have favoured *sh2*-sweetcorn as compared to standard (*su*) and sugary enhanced (*se*) sweetcorn as it is characterised by higher sugar content (Brecht *et al.*, 1990; Geeson *et al.*, 1991; Perkins-Veazie *et al.*, 1994), longer harvest period and greater shelf life (Tracy, 1997). *Sh2*-sweetcorn cobs have approximately 80% higher kernel moisture content and a crispier texture than other sweetcorn genotypes (Tracy, 1997; Ortiz de Bertorelli *et al.*, 2002).

Postharvest life of sweetcorn can be extended by using controlled atmosphere (CA) (Deak *et al.*, 2007), as it suppresses development of pathogens and can inhibit sugar reduction in sweetcorn kernels (Riad *et al.*, 2003; Kader, 2004; Brecht, 2006). Furthermore, storage at low temperatures after harvest and/or under CA storage conditions, are essential for the maintenance of important chemical and physical characteristics of supersweet sweetcorn. It is said that high levels of CO<sub>2</sub> and low levels of O<sub>2</sub> achieved through CA maintain sugar content which is the major quality parameter of sweetcorn after harvesting (Riad and Brecht, 2001). Generally, it is suggested that storage under appropriate CA conditions can extend sweetcorn postharvest life up to two weeks (Hardenburg *et al.*, 1986). High sugar content is a desired attribute in sweetcorn (Azanza *et al.*, 1996a).

Perpetuation of high sugar levels during transportation and shelf-life can be achieved when cobs are stored under moderately high levels of CO<sub>2</sub> and/or low levels of O<sub>2</sub> through CA or modified atmosphere packaging (Riad and Brecht, 2001). It has been intimated that at 1°C, sweetcorn can tolerate as low as 0.5 kPa O<sub>2</sub> and up to 25 kPa CO<sub>2</sub> for two weeks (Riad and Brecht, 2003).

Little research has been conducted on elucidating quality changes occurring in UK-grown cultivars (cvs.) even though there is conventional wisdom that the origin of fruits and vegetables is an important factor that affects their quality and marketability. Despite research showing that quality of *sh2*-sweetcorn can be maintained for up to two weeks under appropriate CA conditions (Riad and Brecht, 2003; Brecht, 2006), the effects of CA on quality of newly developed UK-grown sweetcorn is not known. While the biochemical and textural alterations occurring in postharvest life of fresh sweetcorn have been studied to some extent, the length of storage duration that the quality of sweetcorn was evaluated has been rarely extended to reflect the ‘real world’ supply chain. The null hypothesis on the current study (Experiment 1) was that the newly-UK-developed sweetcorn cvs., Bob 1, Bob 5 and Bob 6 have no significantly different quality characteristics compared with other commonly consumed cvs., during 24 days of storage in CA conditions that are commercially applied through MAP. The alternate hypothesis was that the newly-UK-developed sweetcorn cvs., Bob 1, Bob 5 and Bob 6 have significantly different quality characteristics compared with other commonly consumed cvs., during 24 days of storage in CA conditions that are commercially applied through MAP. The reason for the absence of other CA regimes was that these atmosphere conditions were suggested from sources of the sponsors of this PhD, implying that they might be beneficial and easily achieved through appropriate packaging. Furthermore, due to the null hypothesis of the Experiment, it was preferred to compare newly vs. commonly used cvs., rather than the CA conditions used vs. control regime (air storage), as it would be unachievable to carry out an experiment of the size that would be required for both comparisons. Experiment 1, therefore, aimed to elucidate the temporal changes in textural properties and sugars of seven UK-grown *sh2* sweetcorn cvs. (*viz.* Bob 1, Bob 2, Bob 5, 7210, 6800, Prime Plus and Conqueror) held under specific CA conditions (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) for 24 days at 3°C. In addition,



the current study aimed to examine the suitability of the CA applied, as tool for the perpetuation of the quality of newly developed *sh2*-sweetcorn (*viz.* Bob 1, Bob 5 and Bob 6).

## 4.2 Materials and methods

### 4.2.1 Plant material

Seven UK-grown *sh2*-sweetcorn cvs. (*viz.* Bob 1, Bob 5, Bob 6, 7210, 6800, Prime Plus and Conqueror) were grown on sandy soil by Barfoots of Botley Ltd. (W. Sussex, UK) as per standard commercial practice. Seeds of cvs. Bob 1, Bob 5 and Bob 6 were products of Barfoots of Botley Ltd. Seeds of cvs. Conqueror and Prime Plus were bought from Syngenta (Cambridge, UK) and cvs. 6800 and 7210 from Abbott and Cobb (Caldwell, US). All plants were harvested on the 20<sup>th</sup> September 2007, considering the specifications given from the companies. In particular the maturity point for cv. 6800 is 73 days, for cv. 7210 and Prime Plus is 78 days. Cobs of cv. Conqueror (late maturing) were considered ready to be harvested when the liquid of the kernels was creamy and not watery. However, cvs. Bob 1, Bob 5 and Bob 6, as well as the other cvs. were harvested when the moisture content of kernels was reaching 75%, which is considered to be the peak eating maturity point. That maturity point was also used to ensure the same level of maturity for all the cobs harvested, as the specifications given from the seed suppliers varies depending on the geographic location. The correct maturity point has also been described in Section 2.6. However, it was not defined by measuring the moisture content of kernels. Besides, Azanza *et al.* (1996b), demonstrated that specifically *sh2* cvs., had their maximum sugar concentrations at different moisture levels and therefore moisture content is not appropriate indicator for peak harvest maturity. Actually, it was defined by people trained to recognise the characteristics of peak maturity point (as defined in Section 2.6), involving: presence of kernels and milk line near the tip of the cobs, absence of gaps between kernels, the turning of silk colour to brown, infusion of milky liquid upon kernel puncturing and evaluation of

the visual appearance of kernels which should be attractive (plump). The people (also growers employed at Barfoots of Botley Ltd) which harvested the cobs examined in the experiment were not only trained to recognise the optimum horticultural maturity stage to harvest at, but were also aware of the importance of selecting all cobs on their peak maturity point in order to ensure consistency in the experiment and therefore avoid invalid interpretation of the results derived from all analyses.

Sound cobs [ $n = 7$  (cvs.)  $\times$  6 (outturns)  $\times$  5 (replicates) = 210] were cooled and packed in polystyrene boxes, then couriered to Cranfield University and cooled within 3h. Cobs with husks still intact were transferred to nets suitable for storing vegetables and subsequently stored under CA (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) at  $3 \pm 1^\circ\text{C}$  for 24 days. The specific CA conditions were based on values widely used in commercial environments, in order to examine whether they preserved cob quality.

#### **4.2.2 Controlled atmosphere storage and sample preparation for subsequent analyses**

The experiment was a completely randomised design and the methodology of the required CA conditions is described in Section 3.4. Five cobs per cv. were selected randomly at each outturn (0, 3, 7, 11, 19 and 24 days) for subsequent analyses.

Sweetcorn cobs were removed from storage and husks removed. From the middle section of each cob approximately 20 g of intact kernels were prised off carefully with a sharp knife attempting to avoid damage or stress-reaction of kernels. Ten grams of kernels that were covering the bottom surface of the Kramer Shear Cell were used for textural measurement. The amount of kernels used for the textural measurements were chosen considering that this is an average mouthful portion. The remaining kernels were snap-frozen in liquid nitrogen and then stored at  $-40^\circ\text{C}$ . Frozen tissue was weighed and then freeze-dried and prepared to fine powder for physiological (textural) and biochemical (sugars) measurements as described in Chapter 3.

**Table 4.1:** Effects of storage time and/or CA in moisture content, sugars and texture properties.

Cultivars	°C	Days of storage	CA regimes	M.C. <sup>1</sup> (%)	Sugars	Textural properties	References
Prime time	5	14	2%O <sub>2</sub> -10% CO <sub>2</sub>	n/a	↓ over time (in CA higher levels)	n/a	Riad <i>et al.</i> , 2003
Prime time	5	10	2%O <sub>2</sub> -(0, 15, 25% CO <sub>2</sub> )	n/a	Maintained for 2 weeks in CA	n/a	Riad and Brecht, 2003
Prime time	5	14	2%O <sub>2</sub> -(0, 15, 25% CO <sub>2</sub> )	n/a	Maintained for 2 weeks in CA	n/a	Riad and Brecht, 2003
Prime time	1 & 5	10	2%O <sub>2</sub> -(10, 20% CO <sub>2</sub> )	n/a	↓ with time and Temp. but maintained in higher levels than in air storage	n/a	Riad and Brecht, 2001
How sweet it is	6	5		81.4±1.4	↓	n/a	Zhu <i>et al.</i> , 1992
7210	1	9		n/a	↓ over time	n/a	Brecht <i>et al.</i> , 1990
Sucro	1, 4 & 7	7		n/a	No s. d. <sup>2</sup>	n/a	Olsen and Jordon, 1989
Unknown	10 & 20	16	n/a	↓ with time and Temp. <sup>3</sup>	-	↑ with time and Temp. (Rupture energy)	Deak <i>et al.</i> , 1987

<sup>1</sup>Moisture content, <sup>2</sup> Significant difference, <sup>3</sup>Temperature

Arrow indication: ↑ Increase; ↓ Decrease

n/a: not applicable

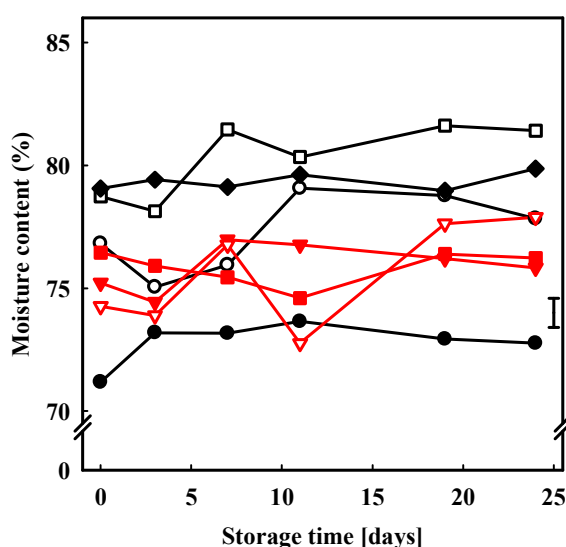
## 4.3 Results and discussion

### 4.3.1 Moisture content of kernels

Generally, decrease in moisture due to respiration of fruits and vegetables results in product deterioration (Rickman *et al.*, 2007a). Kernel moisture content is closely related to kernel length, width, surface area, thickness and other physical properties of dried corn kernels (Karababa and Coşkuner, 2007) and occasionally linked to texture properties and sugar transformation into starch (Szymanek *et al.*, 2003). Relevant studies and the highlights of their results are presented in Table 4.1.

The kernel moisture content of the sweetcorn samples stored was between 71-83% (Figure 4.1). Significant difference was found between the mean moisture content between cvs. and time. The main reason for moisture loss is transpiration (Showalter, 1963) and occurs primarily in the husks which in turn incur moisture loss from kernels and cobs in the form of water vapour (Handerburg *et al.*, 1986). Thus, it can be assumed that the husks of the cobs examined in that experiment were more tolerant to moisture loss in the applied storage temperature and CA conditions rather than in the case that cobs were not covered with husks. If the moisture loss from the husks had not been substituted by water from the kernels and in combination with product respiration, this would be apparent by a decrease in the weight of the cobs (Showalter, 1963). This theory cannot be proved in this experiment as the weight of the cobs was not recorded. In addition to all these factors, and while kernels were cut off with a sharp knife and with every possible effort to minimise damages in kernels by detaching them from their base of the cobs, it is not possible to exclude the eventual water loss from the cut surfaces. On the other hand the indirect effects of respiration and therefore conversion of dry matter into CO<sub>2</sub> and release of H<sub>2</sub>O, is not insignificant in products with high respiration rates (see section 2.7.2) like sweetcorn. However, there was a significant increase in the overall mean moisture content during cold storage; this result is in agreement with previous work that showed a significant increase in moisture content of kernels during the postharvest life of three sweetcorn cultivars (Vigneault *et al.*, 2006). According to Figure 4.1, after some days of storage cvs. Bob 5 and Conqueror, had a significant increase in their moisture content. The same increase had been observed by

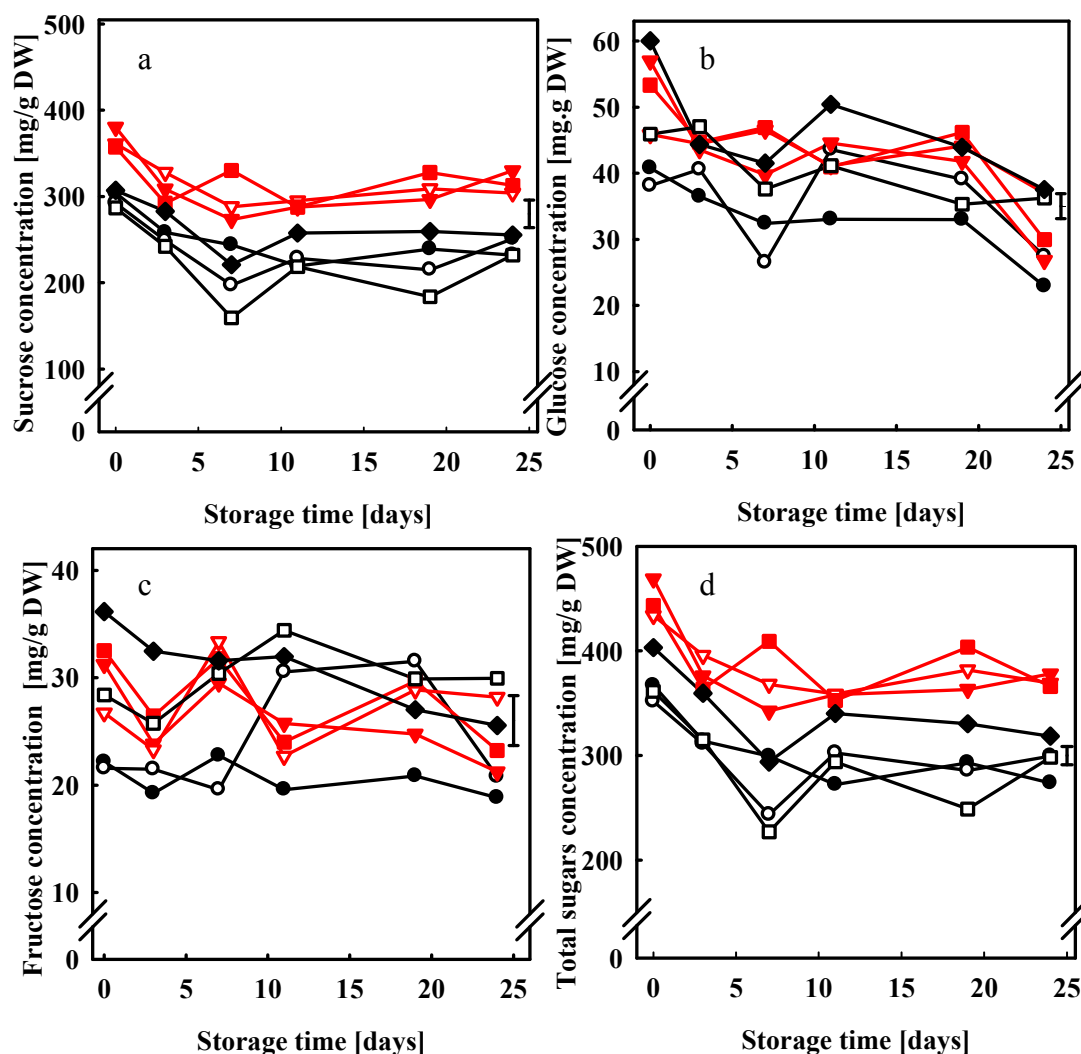
Olsen *et al.*, (1990). The authors stated as a possible reason for this increase the potential condensation of sugars to polysaccharides and consequently the release of H<sub>2</sub>O, but they had not analysed sugars individually to support this theory. In the current study where sugars were analysed individually (Section 4.3.2), there was no evidence that the reason of the increased moisture content was the condensation of sugars to polysaccharides. Significant increases in moisture content shown in Figure 4.1 can be explained by transpiration of water from the cobs to the kernels. Besides, the possibility of water uptake from the atmosphere is minimised as there is not such an indication in the other cvs. Furthermore, the potential that the stomata close after a significant loss of moisture (cv. Bob 5, days 5-10), in combination with water transpiration to kernels contributes to the idea that moisture accumulated in the kernels during the 10<sup>th</sup> to 15<sup>th</sup> day of storage for cv. Bob 5. In addition, the mean of moisture content measured during the whole experiment was highest for cv. Conqueror compared to the other cvs. and moisture content values were lowest for cv. 6800 (Figure 4.1), probably due to genetic variation. Considering that moisture content is commonly used to test sweetcorn quality and that a drop of kernel moisture below 75% results in a decline of flavour, texture and quality (Ghorpade *et al.*, 1998) cv. 6800 is suggested to have the lowest quality –but not low- compared to the other cvs.



**Figure 4.1:** Moisture content of seven *sh2* sweetcorn cvs. (●) 6800, (○) 7210, (▼) Bob 1, (▽) Bob 5, (■) Bob 6, (□) Conqueror, and (◆) Prime Plus stored at 3°C during 24 days. The bar indicates the l.s.d. of genotype and storage time.

### 4.3.2 Sugars

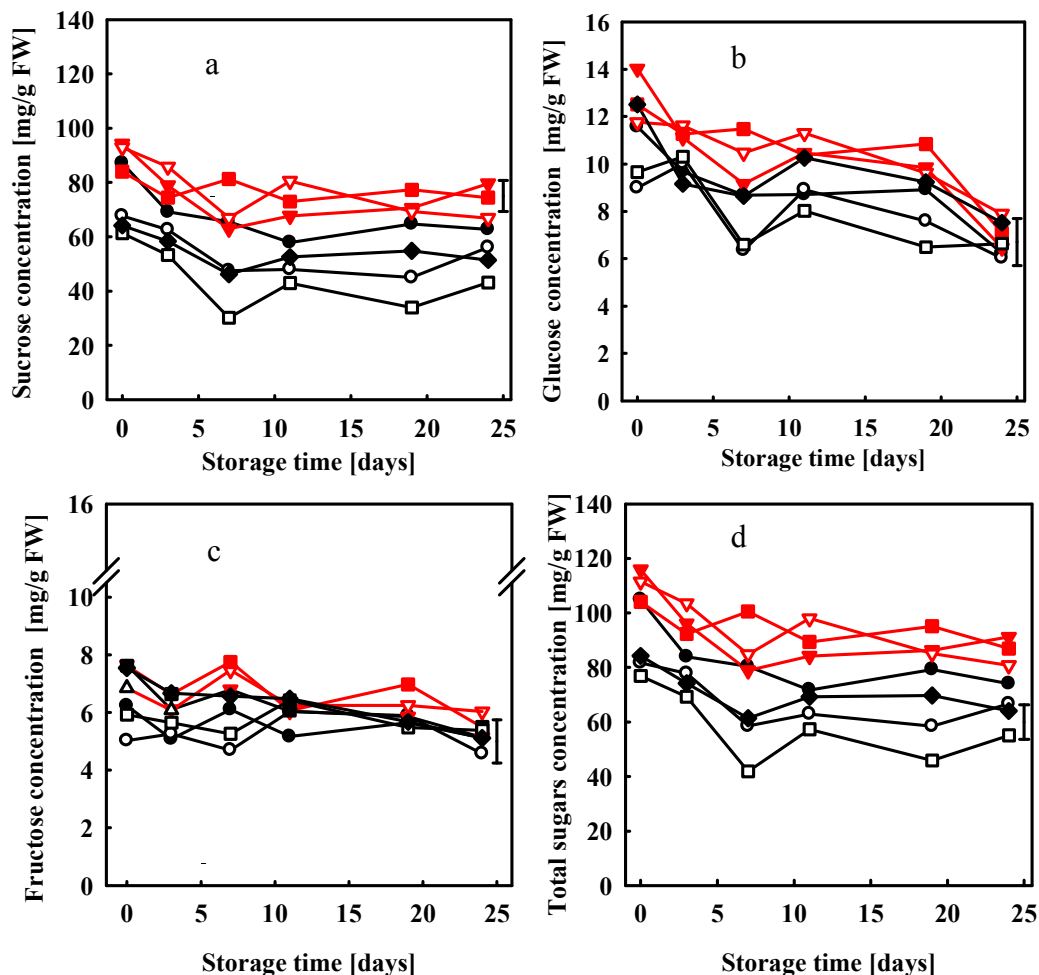
Total sugars were higher on both a fresh and dry weight basis in cvs. Bob 1, Bob 5 and Bob 6 as compared to cvs. Conqueror, Prime Plus, 6800 and 7210 (Figures 4.2-4.3). Total sugars concentration decreased during storage (Figures 4.2-4.3) and this was in agreement with previous studies (Ghorpade *et al.*, 1998; Riad and Brecht, 2001). Brecht *et al.* (1990) who studied sugar content of cv. 7210, found that it was *ca.* 8% on fresh weight basis. The amount of sugars on fresh weight basis of cv. 7210 was also 8% at the first day of the experiment in the present study (Figure 4.3). Total sugar content on a dry weight basis, of which sucrose was the main component (*ca.* sucrose 80.3%, glucose 11.9% and fructose 7.8%), was significantly different between cvs. and was highest for cvs. Bob 1, Bob 5 and Bob 6 (Figure 4.2). Sucrose concentration on dry weight basis showed a general propensity to decline at the beginning of storage and then stabilised thereafter (Figure 4.2). The reason that in general sugar content of Bob cvs. was higher than that of other cvs., can be explained by the combination of the initially high sugar content of Bob cvs. and the ability of Bob cvs. to retain sugars, as result of their genetical characteristics. This assumption becomes convincing as the first half of the storage period, total sugars are more clearly higher than in the second half (Figure 4.2). Azanza *et al.* (1996b) they also concluded that the major effects to sugar content, endosperm firmness and textural characteristics of sweetcorn derived from genetic variation. The main source of variation between quality characteristics of sweetcorn including moisture content were also the genotypic differences among 24 *sh2* cvs. that Wong *et al.* (1994), studied. The variation that authors observed was so extensive that indicate that concentration of individual and total sugars were strongly affected by the allelic variation at other loci.



**Figure 4.2:** Mean concentration (mg/g DW) of sucrose (a), glucose (b), fructose (c) and total sugars (d) on dry weight basis, derived from the sum of values measured in seven *sh2* sweetcorn cultivars (●) 6800, (○) 7210, (▼) Bob 1, (▽) Bob 5, (■) Bob 6, (□) Conqueror, and (◆) Prime Plus stored at 3°C during 24 days. The bars indicate the l.s.d. of combined cv. and storage time.

Means of glucose and fructose concentration declined regardless of genotype. Time of storage and cv. as well as their interaction are factors that influenced the sweetness of the sweetcorn cobs examined on fresh weight basis. In particular, and in agreement with the results referring to sugar content, cvs. Bob 1, Bob 5 and Bob 6

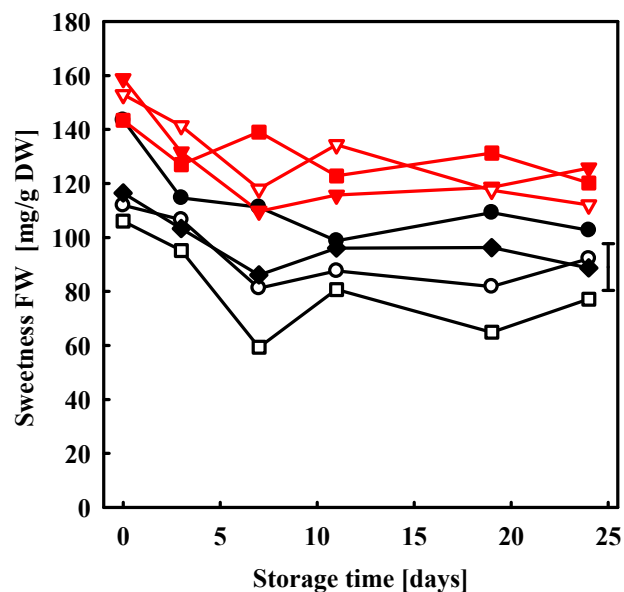
would be deemed by consumers as being sweeter than the other cvs. and sweetness appeared to decline over time (Figure 4.4).



**Figure 4.3:** Mean concentration (mg/g FW) of sucrose (a), glucose (b), fructose (c) and total sugars (d), derived from the sum of values measured in seven *sh2* sweetcorn cultivars (●) 6800, (○) 7210, (▼) Bob 1, (▽) Bob 5, (■) Bob 6, (□) Conqueror, and (◆) Prime Plus stored at 3°C during 24 days. The bars indicate the l.s.d. of combined cultivar and storage time effect for sucrose, glucose, fructose and total sugars.

Sweetness was calculated as suggested by Kader (2008) (see Section 3.7.2.1) and revealed that in contrast to the results for total sugars, cv. Prime Plus appeared to be sweeter than cv. 7210, as it has higher concentration of glucose and sucrose that are sweeter sugars (Figure 4.4). However, generally sweetness is closely linked to the non-reducing fraction of sugars (sucrose) content in kernels (Reyes *et al.*, 1982).

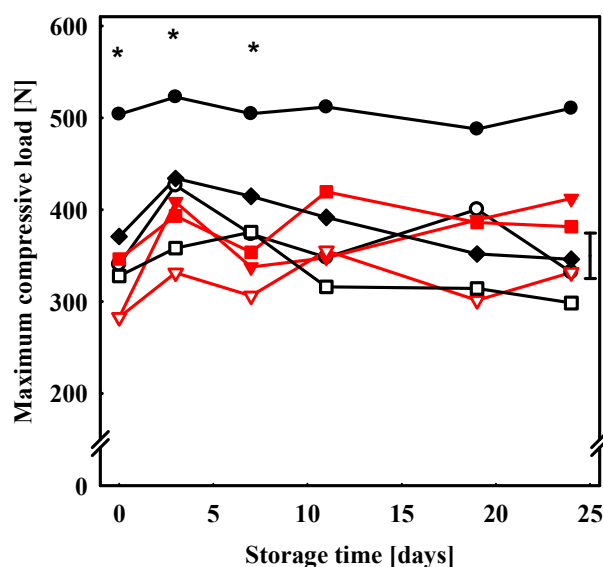




**Figure 4.4:** Mean sweetness derived from the sum of values measured in seven *sh2* sweetcorn cultivar (●) 6800, (○) 7210, (▼) Bob 1, (▽) Bob 5, (■) Bob 6, (□) Conqueror, and (◆) Prime Plus stored at 3°C during 24 days. The bar indicates the l.s.d. of combined cultivar and storage time effect for sweetness.

### 4.3.3 Texture

There was a main effect of genotype and storage duration on the maximum compressive load measured by means of Kramer Shear Cell in the sweetcorn cobs stored under CA conditions (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) (Figure 4.5). Kramer Shear Cell is used to measure shear force but in this experiment maximum compressive load which is related to the force necessary to break the kernels, is reported. Maximum compressive load was significantly lower at day 0 (harvesting day) than on any other day. It has also been shown that after an initial increase in maximum compressive load the texture was maintained during storage.



**Figure 4.5:** Maximum compressive load (N) of seven *sh2* sweetcorn cvs. (●) 6800, (○) 7210, (▼) Bob 1, (▽) Bob 5, (■) Bob 6, (□) Conqueror, and (◆) Prime Plus stored at 3°C during 24 days. \* indicates that more than one values were above instrument limit of 525 N. The bar indicates the l.s.d. of combined cultivar and storage time effect.

Generally, the maximum compressive load of cv. 6800 during storage was significantly higher than for all the other cvs. (Figure 4.5), and may not only be explained by genotype, but also by possible differences in horticultural maturity of cvs. when harvested (Hale *et al.*, 2004). In the present experiment possible differences in horticultural maturity were minimised as much as possible (see Section 4.2.1) so that elucidation between genotypes would be accurate. Cv. 6800, had the lowest moisture content and the highest maximum compressive load of all cvs., which was expected as moisture content has a strong effect on the mechanical properties of kernels. In fact, it has been reported that at lower moisture content, the strength properties of the kernels are increased (Szymanek *et al.*, 2003). Also, the mean of maximum compressive load on the 3<sup>rd</sup> day of the experiment was higher than on day 0 (Figure 4.5). There was a slight association between moisture content and maximum compressive load, since slightly lower average moisture content on the third day led to tougher kernels. Considering, that storage conditions commonly used for sweetcorn aim to retain quality characteristics it can be said that the CA conditions used in combination with storage temperature at 3°C, were appropriate in terms of texture.

#### 4.3.4 Controlled atmosphere and sweetcorn perishability

It has been suggested that reduced O<sub>2</sub> and elevated CO<sub>2</sub> levels in storage atmosphere of sweetcorn is not always beneficial (Riad and Brecht, 2003). In more detail, authors stated that respiration rate of sweetcorn in CA (2 kPa O<sub>2</sub> and 25 kPa CO<sub>2</sub>) was higher than air storage while 2 kPa O<sub>2</sub> and 0 kPa CO<sub>2</sub> or 2 kPa O<sub>2</sub> and 15 kPa CO<sub>2</sub> did not affect the respiration of sweetcorn compared to air control. Furthermore, CA conditions of 2 kPa O<sub>2</sub> and 15 kPa CO<sub>2</sub> were shown to be beneficial for maintaining high sugars levels without induction of fermentative metabolism. According to Olsen (1999), a beneficial combination of gases during postharvest life of sweetcorn ranges between 2-4 kPa O<sub>2</sub> and 5-10 kPa CO<sub>2</sub> at 0°C; yet, in the current work the temperature and the concentration of gases were higher. It also has been suggested that CO<sub>2</sub> levels greater than 10 kPa can be injurious to sweetcorn (Ghorpade *et al.*, 1998). The suitability of the CA used herein (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) at 3°C was demonstrated as changes in texture, sugar and moisture content when significant were not in unacceptable levels in terms of quality. Furthermore, no disease or symptoms caused by anaerobic conditions were observed for 24 days contributing to the idea of the suitability of the CA used.

#### 4.4 Conclusions

The current study demonstrated that the combination of 8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub> at 3°C was suitable for maintaining sugar content and firmness of sweetcorn for 24 days and that the retention of sucrose was affected by genotype. Maximum compressive load was also strongly dependent on the genotype. The null hypothesis was rejected and the alternate hypothesis was confirmed. Therefore the newly-UK-developed sweetcorn cvs., Bob 1, Bob 5 and Bob 6 have significantly different quality characteristics than other commonly consumed cvs., during 24 days of storage in CA conditions that are commercially applied through MAP. Actually, the newly bred cvs. (Bob 1, Bob 5 and Bob 6) had better quality compared to the other cvs. examined in terms of sugars and texture. According to the results from the current study, genetic differences are a great

source of variation in determining the major quality characteristics of sweetcorn (moisture content, dry mater, sugar content and texture).

## 5. CHAPTER FIVE

### Effects of genotype, origin, storage period, format and storage temperature on the texture and sugar content of sweetcorn cultivars

#### 5.1 Introduction

The fresh sweetcorn market has seen an important development in the past few years. Major quality parameters such as sugar content and texture-related parameters of different genotypes of sweetcorn have been the subject of much research (Azanza *et al.*, 1996a; Azanza *et al.*, 1996b; Barrett *et al.*, 2000). On the other hand, growing conditions and environment such as soil type and temperature are preharvest factors that also have significant effects on quality attributes of sweetcorn (Ghorpade, 1998; Tracy, 1997). Furthermore, the influence of storage temperature on important chemical characteristics of sweetcorn has been studied, suggesting that storage temperature closer to 0°-2°C is more beneficial to the postharvest life of sweetcorn than higher temperatures (Brecht, 2002; Riad, 2004).

In this Chapter, the 2<sup>nd</sup> and the 3<sup>rd</sup> Experiment conducted are described. The null hypothesis of Experiment 2 was that the factor cv. and storage packaging during transportation do not have significant effects on sugar content of *sh2*-sweetcorn and the alternate hypothesis that sugar content of *sh2*-sweetcorn is affected by the cv. and the type of storage packaging. On the other hand, the null hypothesis of Experiment 3 was that storage temperature, presence of husks during storage and origin of sweetcorn cobs does not influence the sugar content and texture while according to the alternate hypothesis these attributes are affected by storage temperature, presence of husks during storage and origin of the cobs. In the current work, the effects of different genotypes, origin (Senegal vs. USA), storage temperature (6°C vs. 16°C), storage time and cob format [with husks (window stripped) and without husks (naked)] were studied. Oluwatola *et al.* (1998) compared the tenderness of cobs with and without husks and

found that cobs stored with husks were more tender and also stated negative correlations between moisture content and hardness of kernels. According to their data, it was shown that husks provided a limited protection to textural characteristics of green field maize. However, the authors referred to green field maize and not *sh2*-corn. The presence of husks affects the moisture content of kernels and in turn the sugar content of kernels (see Section 2.3). In addition as it is explained in Section 4.3.1, respiration rate and therefore several biochemical characteristics can be affected by moisture. Thus and knowing these indications about green field maize, in this study it was attempted to elucidate the effects of husks in the texture (in terms of firmness) and other quality attributes in popularly consumed supersweet sweetcorn cvs., when stored in different temperatures. In particular, the objectives of the present study were: (1) to compare several kernel carbohydrate components of various *sh2* cultivars for high levels of sugar (2) to monitor the effect of two postharvest temperatures on the concentration of these components; (3) to clarify the effects of the presence of leaves in the cultivars vs. naked ones; (4) to examine the differences in the concentration of sugars and firmness on cvs. of different origin (5) to look at the effects of the above factors on storage life.

## 5.2 Materials and Methods

For the purposes of the present study two experiments were conducted. Determination of O<sub>2</sub> and CO<sub>2</sub> concentration in the internal atmosphere of sweetcorn packages was quantified as described in Section 3.4.2. Maximum compressive load was recorded as described in Section 3.6.2, after removing husks from the window stripped cobs. Before analysis of sugars, all cobs were cut and the kernels removed from the cobs. Kernels were then snap-frozen with liquid nitrogen and stored at -40°C for subsequent analysis. Sugar content of the kernels and statistical analyses were determined as described in Chapter 3.

### 5.2.1 Experiment 2

Sweetcorn cobs harvested the same day were couriered on 20/02/2008 to Cranfield University and immediately cut and processed for analysis of sugar content. The cobs were separated into four groups as shown in Table 5.1:

**Table 5.1:** Design of experiment 2

Format	Packaging	Cultivar	Origin	Abbreviated name	Number of replicates
Naked	Bags	Prime Time	Senegal	Tesco-Sen.	n=5
Naked	Bags	Garrison	USA	Garrison-Packed	n=5
Husked	Bushel Boxes	Garrison	USA	B.B. Garrison	n=5
Naked	Bags	Prime Time	Senegal	Sen.PrimeTime	n=5

### 5.2.2 Experiment 3

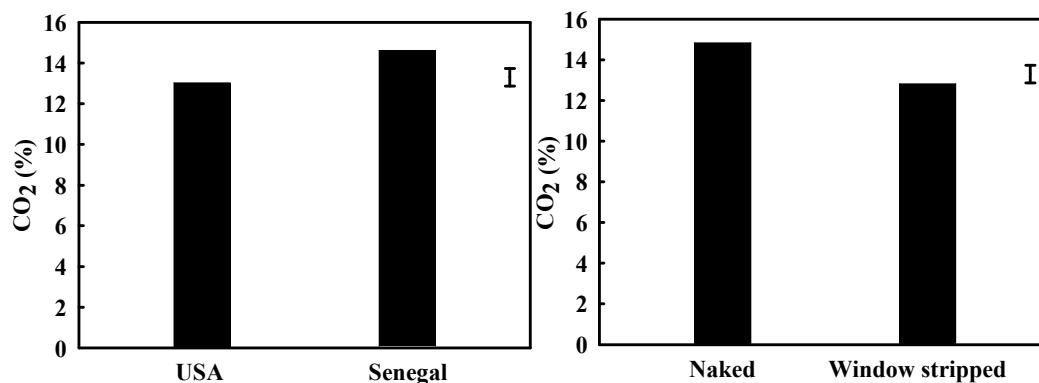
For Experiment 3, one cv. (Garrison) from two different origins (Florida-USA and Senegal) was studied. Sweetcorn cobs were harvested on the same day, packed in polypropylene bags and couriered to Cranfield University where they were stored in two cold rooms set up at 6°C and 16°C, respectively [n=80, (n=40 stored at 6°C + n=40 stored at 16°C)] for each origin and format. Thus, total cobs stored was n=320 (window stripped and naked). The 02/04/08 was considered as day 0 of the experiment. Four cobs of each origin and format were taken for texture analysis every second day for 12 days in total [kernels tested were from all over the cobs (n=12/cob)]. Respiration gases and sugar content were also determined in the current experiment.

## 5.3 Results and discussion

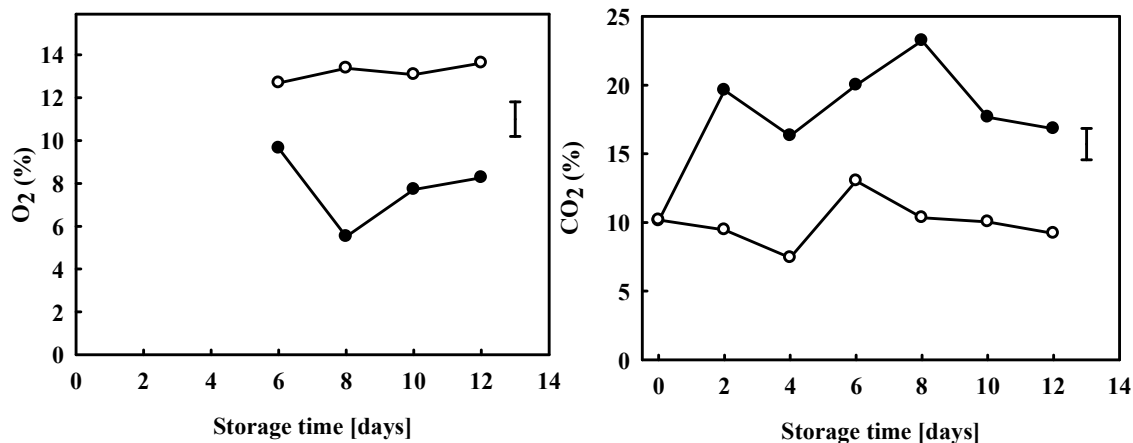
### 5.3.1 Gases (O<sub>2</sub> and CO<sub>2</sub>)

The optimum CA storage conditions for sweetcorn have been reported to be 15% CO<sub>2</sub> and 2% O<sub>2</sub> (Brecht, 2002). In the present study the concentration of O<sub>2</sub> was greater than the recommended concentration (Figure 5.2). Generally, the results obtained from gas measurements showed that there were significant differences in CO<sub>2</sub> concentration between naked and window stripped sweetcorn. In particular, CO<sub>2</sub> concentration was higher in packages with naked cobs than in packages with window stripped cobs. Furthermore, the origin of the cobs also influenced CO<sub>2</sub> concentration.

Cobs originating from Senegal had higher CO<sub>2</sub> concentration in the packs than cobs originating from USA (Figure 5.1).



**Figure 5.1:** Concentration of CO<sub>2</sub> (%) measured in cobs originating from USA and Senegal and concentration of CO<sub>2</sub> measured in window stripped and naked sweetcorn (%) as derived from the mean values over 12 days of storage. The bars indicate the l.s.d. of origin and format respectively.



**Figure 5.2:** Concentration of O<sub>2</sub> (%) and of CO<sub>2</sub> (%) measured in sweetcorn packs stored at 6°C (○) and at 16°C (●). The bars indicate the l.s.d. of combined storage temperature and storage time.

Storage temperature significantly affected both O<sub>2</sub> and CO<sub>2</sub> concentrations. At 6°C, CO<sub>2</sub> concentration was lower and O<sub>2</sub> concentration was higher than in sweetcorn packs stored at 16°C. In addition, the concentration of O<sub>2</sub> changed significantly at 16°C over storage time, especially from day 6 to day 8. It is worth noting that development of



pathogens was increased in cobs stored at 16°C. The conclusions above are also verified from the results of CO<sub>2</sub> that are opposed to those of O<sub>2</sub> (Figure 5.2). Generally it is believed that in both controlled and modified atmosphere, elevated levels of CO<sub>2</sub> and low levels of O<sub>2</sub> are beneficial for the retention of sugar concentration while delaying the development of pathogens (Aharoni *et al.*, 1996; Riad, 2004).

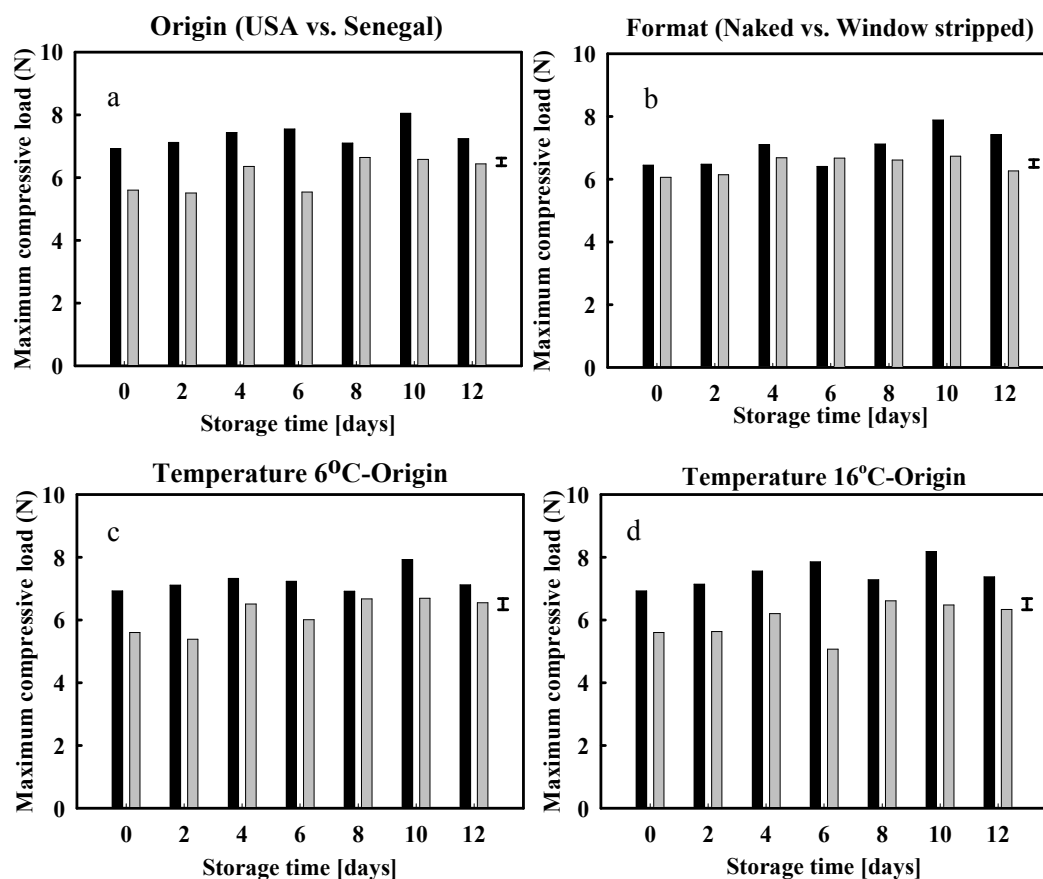
### 5.3.2 Maximum compressive load

Variation in texture-related parameters due to different genotypes and different locations of growth has been previously studied (Tracy, 1997). In the current work, maximum compressive load was found to be lower for cultivars originating from Senegal in comparison with cobs originating from USA when stored at 6°C or 16°C (Figure 5.4). Kramer *et al.*, (1949) had reported that changes in texture might be related to the chemical composition of kernels (Kramer *et al.*, 1949).

Window stripped cultivars are likely to be less firm than the naked ones and this observation was more noticeable on the last days of the experiment. Cultivars grown in USA at 6°C storage temperature appeared to have constant firmness over time until day 6, in contrast to Senegalese-grown cobs. It should be noted that the microbial growth in sweetcorn stored at 16°C, was observed in approximately 15% of the kernels at day 4 and was elevated at a rate of *ca.* 10% every two days. Figure 5.3 displays the fungal problems observed during Experiment 3.



**Figure 5.3:** Fungal problems in Senegalese sweetcorn cobs stored at 16°C, after 6 days.



**Figure 5.4:** Maximum compressive load measured in (a) sweetcorn originating from USA (■) and Senegal (■), (b) in naked (■) and window stripped (■) cobs and in sweetcorn cobs originating from Senegal (■) and USA (■) stored at 6°C (c) and at 16°C (d) during storage. The bars indicate l.s.d. of combined storage time and origin or format for plots (a) and (b) respectively. In plots (c) and (d) the bars indicate the l.s.d. of combined storage time, origin and temperature.

### 5.3.3 Sugar content

#### 5.3.3.1 Experiment 2

Sugar content between cobs examined, varied significantly. Garrison cv. was shown to have higher sugar content than cv. Prime Time. Sugar content of naked cobs from cv. Garrison did not differ significantly from sugar content of fully husked Garrison cobs. However, not all the individual sugars measured had greater

concentration in cv. Garrison, suggesting that not only total sugar content is affected by genotype but also the distribution of sugars (Table 5.2).

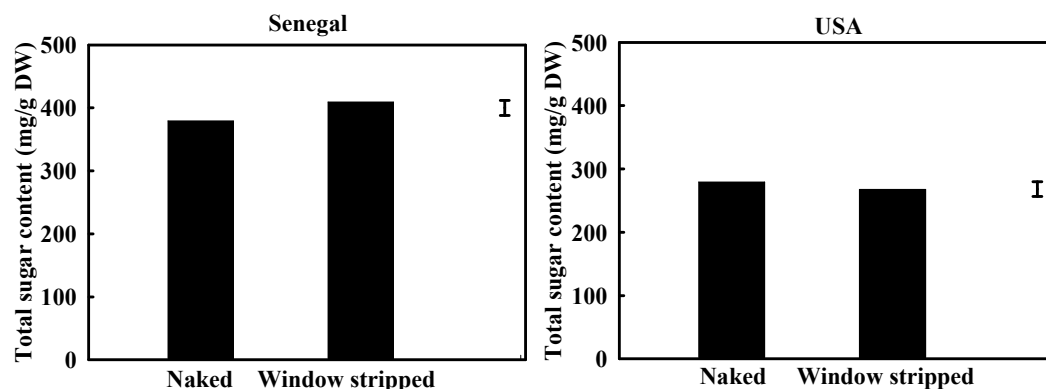
**Table 5.2:** Sugar concentration (Sucrose, glucose and fructose) of sweetcorn cobs originating from Senegal and USA, on DW and FW basis.

	Cultivar	Fructose	Glucose	Sucrose	Total sugars
FW	Tesco-Sen.	5.138a	8.46b	49.7b	63.3c
	Garrison-				
	Packed	4.966a	10.2a	67.6a	82.8a
	B.B.Garrison	4.803a	9.4a	61.5a	75.7ab
	Sen.Prime				
	Time	3.651b	7.45c	59.7a	70.8bc
	l.s.d. (P=0.05)	0.5637	0.83	7.9	8.6
DW	Tesco-Sen.	25.05a	41.18b	241.3c	307.5c
	Garrison-				
	Packed	22.9a	46.98a	311.2a	381.1a
	B.B.Garrison	22.72a	44.33ab	289.1ab	356.2ab
	Sen.Prime				
	Time	16.95b	34.59c	276.9b	328.5bc
	l.s.d. (P=0.05)	3.126	4.215	33.58	37.17

### 5.3.3.2 Experiment 3

Sucrose was the predominant sugar found in the cobs analysed, in agreement with previous works (Ferguson *et al.*, 1979; Tracy, 1997; Yusef and Juvik, 2001). Total sugar concentration of sweetcorn cobs originating from Senegal was significantly higher than of cobs originating from USA while significant differences regarding the format were observed (naked vs. window stripped) (Figure 5.5). However, format influenced only the total sugar content of sweetcorn cobs originating from Senegal,

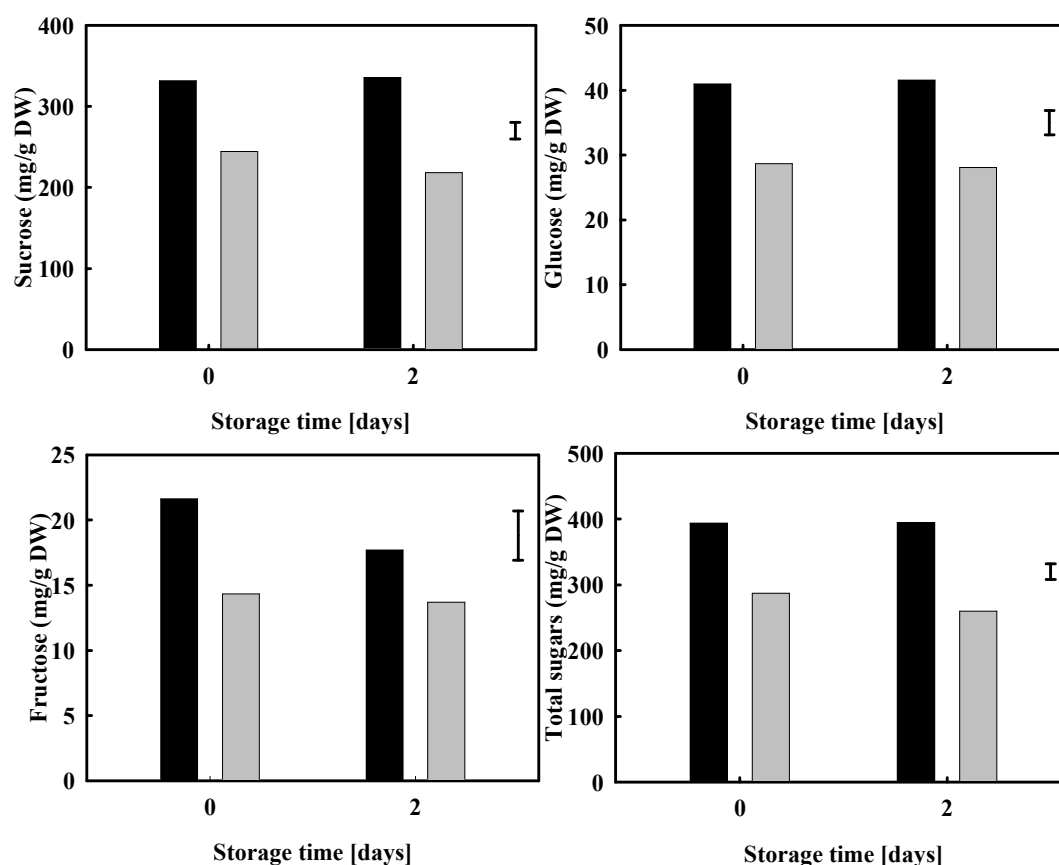
where as presented in Figure 5.5, window stripped cobs had higher sugar content than naked cobs.



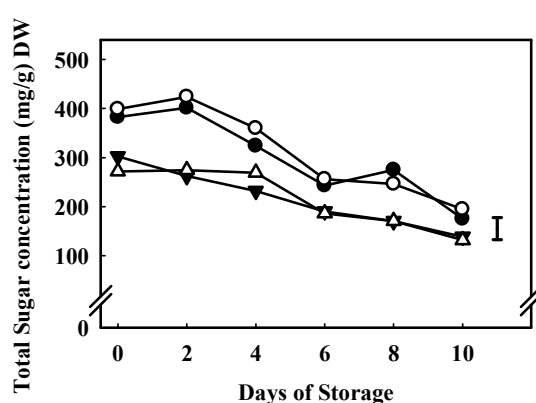
**Figure 5.5:** Total sugar concentration of cv. Garrison originating from Senegal and USA stored as naked and window stripped as derived from the mean values over 2 days of storage at 6°C and at 16°C. The bar indicates the l.s.d. of combined format and origin.

It is worth noting that from the fourth day of storage at 16°C, pathogens developed (not identified) in sweetcorn cobs originating from Senegal (Figure 5.3). Sweetcorn coming from USA also had fungal problems when stored at 16°C, but after 8 days of storage. When stored at 6°C, sweetcorn cobs from Senegal again developed fungi faster but after day 8. Thus, some replicates were lost and consequently statistics for the last days of the experiment (8 and 10) are less valid than for the first experiment due to a lower number of replicates. For the same reason and to validate the statistical analysis, sugar content of cobs stored at 6 and 16°C was compared only for the first two outturns (Figure 5.6). Thus, results were in agreement with Brecht (2002) who stated the beneficial effects of low storage temperature.

On the other hand, total sugar concentration of cv. Garrison originating from Senegal and USA stored as naked and window stripped at 6°C, indicates a decline over 10 days of storage (Figure 5.7). On this Figure (5.7), the effect of format is not apparent as it is displayed in interaction with origin and storage time.



**Figure 5.6:** Sucrose, glucose, fructose and total sugar concentration of cv. Garrison originating from Senegal (■) and USA (■) stored as naked and window stripped over 2 days of storage at 6°C and at 16°C. The bar indicates the l.s.d. of combined storage time and origin.



**Figure 5.7:** Total sugar concentration of cv. Garrison originating from Senegal stored as naked (●), and window stripped (○), and originating from USA stored as naked (▼) and window stripped (Δ) over 10 days of storage at 6°C. The bar indicates the l.s.d. of combined storage time, format and origin.

According to the results, it is indicated that the origin of the cobs had strong effects on sugar content of the cobs examined, probably as result of the preharvest factors influence the postharvest quality of sweetcorn (see Section 2.6). Storage temperature had also great influence on the cobs, as cobs stored at 16°C were deteriorated so rapidly that did not allow comparison with cobs stored at 6°C. Important observations were also those concerning the effects of format of cobs during storage on their sugar content. The same cv. was influenced from the format when was originating from Senegal but not when was originating from USA which also indicates the significance of the interaction of the format of the cobs and their origin.

## 5.4 Conclusions

The null hypothesis of Experiment 2 was rejected as the factor cv. and storage packaging during transportation had significant effects on sugar content of *sh2*-sweetcorn. The null hypothesis of Experiment 3 was partially rejected as storage temperature, presence of husks during storage and origin of sweetcorn cobs influence the sugar content and texture. However, the null hypothesis was only partially rejected as total sugar content of cobs originating from USA was not affected by the format of the cobs during storage. While, format does not seem to always have significant effect on sugar content, the period of storage does, indicating a decline in sugar content over time. However, texture and respiration gases were influenced by the interaction between origin and format. Storage temperature at 6°C appeared to better maintain the quality of sweetcorn in terms of maintaining sugars and retarding microbial growth than storage temperature at 16°C. With the exception of origin, the high variation in the effects of the other factors studied indicates that further research is required. In particular, this variation with the additional loss of experimental samples due to high storage temperature led to the need for other experiments to be carried out (Chapter 6 and 8) in order to clarify the effects of format on sweetcorn quality attributes.

## 6. CHAPTER SIX

### **Textural characteristics and spatial sugar profile of a supersweet sweetcorn stored with or without husks**

#### **6.1 Introduction**

The increase in sweetcorn cob consumption has led to more research into several quality parameters during storage. Sweetness and firmness are the major quality attributes in sweetcorn cobs such that maintaining these is critical. In the fresh sweetcorn market, there is an ongoing dispute over whether sweetcorn cobs sold in a 'naked' (husks having been fully removed) format are preferred by consumers as compared to the window stripped (cobs with a narrow area free of leaves) retail format. Furthermore, transport of sugars in maize from sources to sinks during development has been investigated from several researchers. Deposition of sugars from corn cobs and husks to kernels of normal maize hybrids is considered possible (Andrews *et al.*, 2000). Research on carbohydrate distribution in sugar enhanced sweetcorn suggested that sugar content of kernels at fresh-market maturity stages could be predicted when changes of sugar content in the entire plant are known (Russo *et al.*, 2004). In contrast, biochemical profiling of corn cobs (core) and shank of sweetcorn of *sh2* genotypes during postharvest life is very limited. This deficiency in the literature is surprising given that this knowledge could be helpful in understanding potential uses of core and shanks especially given that some packers are now using sweetcorn waste to generate electricity via anaerobic digestion (e.g. Barfoots of Botley, West Sussex). Understanding calorific value would be useful to enable calculation of potential for energy conversion. The numerous studies that are available about texture of sweetcorn, mainly concern differences between various genotypes, maturity stages and sensory perception (Splitter and Shipe, 1972; Azanza *et al.*, 1996a; Azanza *et al.*, 1996b; Szymanek, 2009), rather than postharvest factors that influence sweetcorn texture. Many studies have also demonstrated the utility of non edible parts of standard corn

such as corn cobs, stalks and shanks for the production of useful products. In particular, corn cobs have been used as animal feeds and can also be used for the production of biofuels and functional chemicals such as fermentable sugars (Tsai *et al.*, 1998; Hang and Woodams, 2001; Mullen *et al.*, 2010).

Regarding the spatial sugar profile of sweetcorn, previous research on *sh2 corn* had indicated that sucrose, glucose and fructose content were not significantly different between lower, middle and upper endosperm of kernels (Doehlert and Kuo, 1990). In contrast, Szymanek *et al.*, (2003) suggested that the higher sugar content of the lower endosperm of the kernels in comparison to the central and the upper endosperm indicates that kernels should be cut off in the basal area of the kernels close to the corn cob. Any spatial gradient in sugar content within the cob is probably due to the differential utilisation of sugars. However, the relationship between sugar content of edible parts (kernels) and non-edible tissues (shank and core) of cobs and differences in sugar content of kernels located in different parts of the cobs has also not been investigated during postharvest life of sweetcorn and may help explain the biochemical changes and possible relationship between format and handling conditions.

Thus, the current study (Experiment 4 and 5) aimed to investigate the temporal and spatial changes in textural properties and sugars of kernels, core and shank of *sh2* sweetcorn cobs held for 10 days at 5°C and the potential for possible relationships between the biochemical profiling of these tissues during postharvest life. The null hypothesis of Experiment 4 was that there is not any interrelationship between sugar and moisture content of kernels, core and shank and the alternate hypothesis was that there is such an interrelationship. The null hypothesis of Experiment 5 was that there is not any interrelationship between texture and sugar and moisture content of bottom, middle and top section of *sh2*-sweetcorn cobs and shank and the alternate hypothesis was that there is such an interrelationship.

Showalter (1963) attempted to confirm that the general trend of maintaining long husks and shanks is essential for the preservation of sweetcorn freshness. Window stripping might affect biochemical changes as Oluwatola *et al.* (1998) stated that it affects moisture content and texture (tenderness) of corn (see Section 5.1). On the other hand, Showalter (1963) finally concluded that husks lose water through transpiration and when removed; water loss occurs in kernels. According to Showalter's study,



denting of kernels from cobs with trimmed shanks [ $< 0.8$  inches (2 cm)] was lower than denting from cobs with shank's length ranging between 3 inches (7.6 cm) and 5 inches (12.7 cm). In contrast, the more intense the trimming the more negative were the effects on husk appearance (induced wilting) and the lower was the weight loss of the kernels during six days of storage. Considering that moisture content of kernels influences the content and metabolism of chemical compounds and the desire of the sponsors of this thesis to elucidate the potential effects of husks in the biochemical profile of *sh2* cvs., the design of Experiment 4 and 5 was developed.

The objectives of this research were (1) to determine the predominant sugar profile of kernels, core and shank of supersweet sweetcorn cobs over time as this could probably reveal information regarding waste utilisation (2) to elucidate the potential that changes in the biochemical profile of non-edible parts of sweetcorn cobs could affect sugar content of kernels as important parameter of consumer acceptance (3) to explore the potential to monitor the flow of sugars by comparing sugar content of kernels and corn cobs in different locations of the cobs (4) to determine if the length of shank and presence of husks might be considered as possible source of variation in chemical and textural attributes of supersweet sweetcorn.

## **6.2 Materials and methods**

### **6.2.1 Plant material**

Two experiments were conducted for the purposes of the present study. Experiment 4 and 5 are described in this Chapter, as both portray spatial profiling of sweetcorn. In particular, Experiment 4 describes spatial profiling in terms of different tissues [shank, and in horizontal axis from the outer to the inner: kernels, core]. Experiment 5 describes spatial profiling in vertical axis (bottom, middle, top). In order to develop the experimental design of Experiment 5, a mini experiment was required as the results of the mini experiments in combination with the results of Experiment 4, would indicate which sources of variation should be elucidated.

**Table 6.1:** Design of experiment 4 and 5

Storage Temperature: $5 \pm 1^\circ\text{C}$	Experiment 4	Experiment 5	
Storage time: 10 days			
Cultivar	6800	Fito 206 & 7210	
Naked - Short shank (2 cm)	n=36	Fito 206: n=16 7210: n=16	
Naked Cobs- Long shank (8 cm)	n=36	n/a	
Window stripped cobs- short shank (2 cm)	n=36	Fito 206: n=16 7210: n=16	
Window stripped cobs- long shank (8 cm)	n=36	n/a	
Total cobs in storage	n=144	n=96	
Samples analysed for sugar content of kernels	n= 126	Bottom: n=216 Middle: n=216 Top: n=216	Total: n=642
Samples analysed for sugar content of core	n= 126	Bottom: n=216 Middle: n=216 Top: n=216	Total: n=642
Samples analysed for sugar content of shank	n= 126	n/a	
Number of replicates for CO <sub>2</sub> content	n=144	n=96	
Weight loss	n/a	n=96	
Cobs tested for their moisture content	n=144	n=96	
Cobs tested for their firmness	n=144	n=96	

#### 6.2.1.1. Experiment 4

For Experiment 4, *sh2*-sweetcorn cv. 6800 was grown in Chichester (UK) by Barfoots of Botley Ltd. (W. Sussex, UK) as per standard commercial practise. The

seeds were bought from Abbott and Cobb (Caldwell, US). Plants were harvested according to specifications given from the company (73 days after planting) on the 6<sup>th</sup> of August 2008, with kernel moisture being *ca.* 75% which is considered to be the peak eating maturity point. Sound cobs (Table 6.1) were air-cooled, packed in polypropylene bags and transferred in polystyrene boxes. Cobs were then couriered to Cranfield University, were cooled on arrival and stored within 3h at  $5 \pm 1^\circ\text{C}$  for 10 days. The experiment was a completely randomised design. Sweetcorn cobs were removed at regular intervals. At each day of measurement (day 0, 2, 4, 6, 8, 10) the husks of window stripped cobs were removed prior to penetrometer measurements and cutting procedure of kernels, core and shank. Day 0 was considered the next day after their transfer to controlled temperature rooms at Cranfield University.

#### **6.2.1.2. Experiment 5**

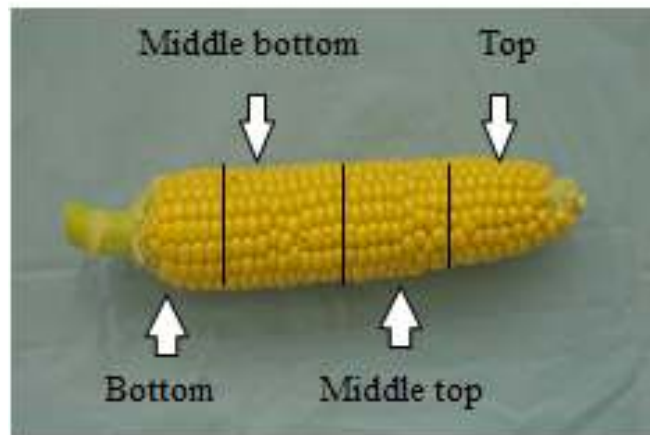
For Experiment 5, core and kernels of sweetcorn cobs of cvs. 7210 and Fito 206 originating from Spain, were examined for their textural characteristics, moisture content and sugar spatial profile (Table 6.1). Cobs were stored  $5 \pm 1^\circ\text{C}$  for 10 days and periodically removed for the purposes of the study at day 0, 2, 4, 6, 8, 10 with starting date the 5<sup>th</sup> of November, 2008.

### **6.2.2 Sample preparation and methodology for subsequent analysis**

The measurement of CO<sub>2</sub> content, moisture content and firmness (maximum compressive load by penetration probe) and the analysis of sugars for both Experiment 4 and 5 were carried out as described in Chapter 3.

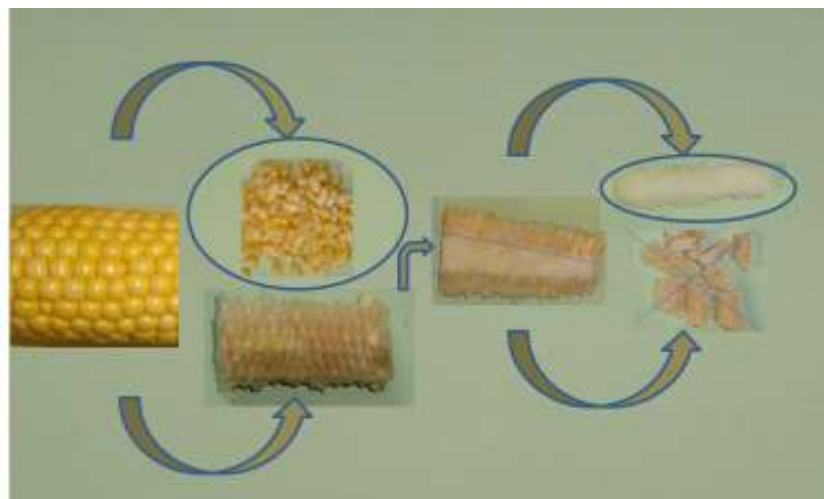
#### **6.2.2.1 Experiment 4**

Sweetcorn cobs were removed from storage and where applicable husks removed. Textural measurements were performed on four sections: bottom, middle bottom, middle top and top (3 replicates for each section = 12 penetrations in total for each cob as shown in Figure 6.1).



**Figure 6.1:** Locations of the kernels chosen to be tested for firmness.

Intact kernels were removed carefully after texture measurements, with a sharp knife attempting to avoid damage. Kernels were then mixed and the final sample was consisted of approximately 20 g of kernels. The procedure applied to obtain kernel and core samples is shown in Figure 6.2. In more detail, after cutting away the ‘woody’ outer portion of the corn cob, it was collected the pith of the cores, consisted by the ring of vascular bundles, which was actually the tissue tested.



**Figure 6.2:** Schematic diagram of kernel and core sample preparation.

The third type of tissue tested was the shank. The term short shank refers to the whole shank (2 cm) without further trimming (see upper cobs of Figure 6.3); while the term long shank refers to the inner part of the shank (2 cm of the whole 8cm) attached to the cob (see position 1 in the cobs of Figure 6.3). Sugar content of kernels and core was also examined spatially at day 0, 4 and 8 ( $n=45/\text{tissue}$ ): bottom vs. middle vs. top as described in next Section (6.2.2.2). Sugar content of long shanks was also examined spatially (see Figure 6.3) at day 0, 4 and 8 [ $n=45$ : bottom vs. middle vs. top]. However, these replicates were mainly used as indicators to organise the experimental design of Experiment 5 and thus, results from this mini-experiment are reported along with the main results of Experiment 5.



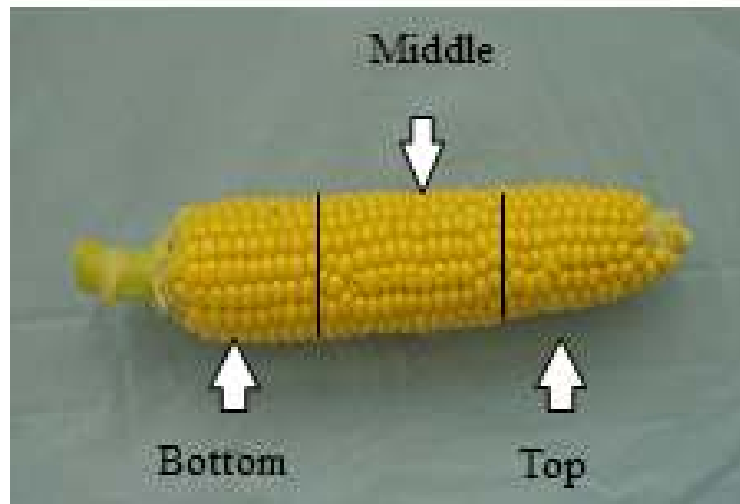
**Figure 6.3:** Sweetcorn cobs with long shank and short shank.

For the main part of Experiment 4, core and kernel samples were taken from all over the cobs and mixed well [Table 6.1:  $n=144/\text{tissue}$ ]. All samples were then snap-frozen in liquid nitrogen and stored at  $-40^{\circ}\text{C}$  for sugar analysis.

#### 6.2.2.2 Experiment 5

In Experiment 5, intact kernels were removed as described in Section 6.2.2.1 at day 0, 2, 4, 6, 8 and 10 of storage. Textural measurements were performed in three parts for Experiment 5: bottom, middle and top (4 replicates for each part=12 penetrations in total for each cob as shown in Figure 6.4). Six samples of each cob were taken: bottom,

middle and top from the core and the kernels (Figure 6.4) under processes described in the previous section, snap-frozen in liquid nitrogen and stored at  $-40^{\circ}\text{C}$  for further sugar analysis.



**Figure 6.4:** Locations of the kernels chosen to be tested for Experiment 4 (*viz.* firmness) and 5 (*viz.* moisture content, firmness and sugars).

## 6.3 Results and discussion

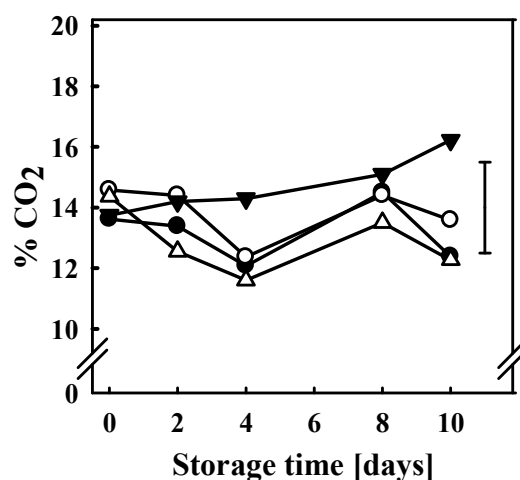
### 6.3.1 $\text{CO}_2$

#### 6.3.1.1 Experiment 4

$\text{CO}_2$  levels in packages containing naked cobs with long shank of cv. 6800 were significantly higher than for window stripped cobs with long shank and naked cobs with short shank at day 10 of the experiment (Figure 6.5). The higher  $\text{CO}_2$  concentration in packs with naked cobs with long shank in comparison to other packs can probably be explained by the size of the surface that respiration occurs. The surface of long shanks is bigger than that of short shanks. On the other hand, the surface of kernels induces greater respiration than the surface of husks. It has been suggested that fatty acids are

one of the main substrates of respiration after sugars, which explains the higher respiration rate of kernels in comparison to husks (Osanyintola, 1995).

Microbial growth would be expected to be lower in packs containing naked cobs with long shank as it is inhibited in elevated CO<sub>2</sub> concentrations that are believed to inhibit respiration and microbial growth (Brecht, 2006). While, this statement has not been tested in the current work, no visual microbial growth was observed in any category of the cobs examined.

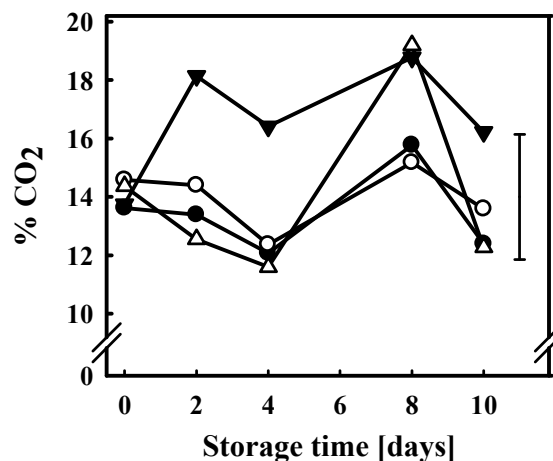


**Figure 6.5:** Means of CO<sub>2</sub> % measured in packages contained naked cobs with short (●) and long shank (▼), window stripped cobs with short (○) and long shank (△) of cv. 6800. The bar indicates least significant difference (l.s.d.) of combined format (naked vs. window stripped), storage time and length of shank.

### 6.3.1.2 Experiment 5

Results showed that there were no significant differences in the mean CO<sub>2</sub> levels in packages containing naked cobs of the cv. 7210 versus those containing window stripped cobs of the same cv. (Figure 6.6). However, CO<sub>2</sub> levels in packages containing naked cobs of the cv. Fito 206 were significantly higher on some days indicating a higher respiration level (Figure 6.6). This may be explained as the result of limited respiration of window stripped cobs due to existence of husks, as they can serve as

barriers against factors provoking deterioration of sweetcorn quality attributes (Deak *et al.*, 1987).



**Figure 6.6:** Means of CO<sub>2</sub> % measured in packages contained naked (●) and window stripped cobs (○) of cv. 7210 and naked (▼) and window stripped cobs (Δ) of cv. Fito 206 during 10 days of storage. The bar indicates the l.s.d. of combined storage time, cv. and format (naked vs. window stripped).

### 6.3.2 Moisture content and weight loss

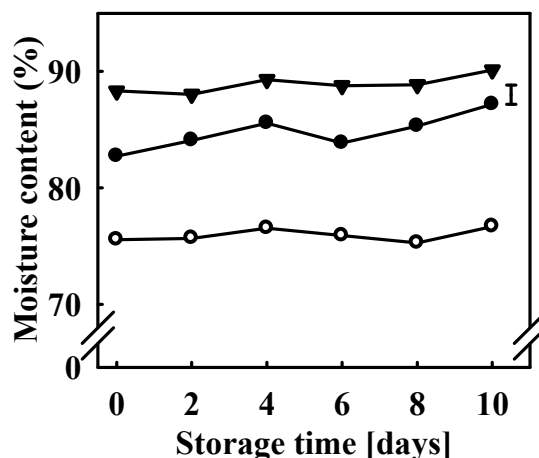
#### 6.3.2.1 Experiment 4

There was a significant increase in the overall mean moisture content of core samples from cv. 6800. However, the interaction of tissue type, storage time and length of shank and the interaction of format, length of shank and tissue type did not significantly affect the moisture content of the samples examined. Length of shank as unique factor of influence did not affect moisture content of tissues examined (see Appendix B). In disagreement, Showalter (1963), found that in cobs with non-trimmed husks (3-5 inches) moisture content decreased steadily in contrast to moisture content of kernels from cobs with trimmed shanks that was retained. However, the cobs that



Showalter examined were standard (*su*) varieties and therefore are deteriorated faster than the supersweet varieties examined in the present study.

Mean moisture content measured during the whole experiment was significantly higher for shank compared to core and kernels. Furthermore, moisture content mean values of kernels were significantly lower compared to core and shank (Figure 6.7).

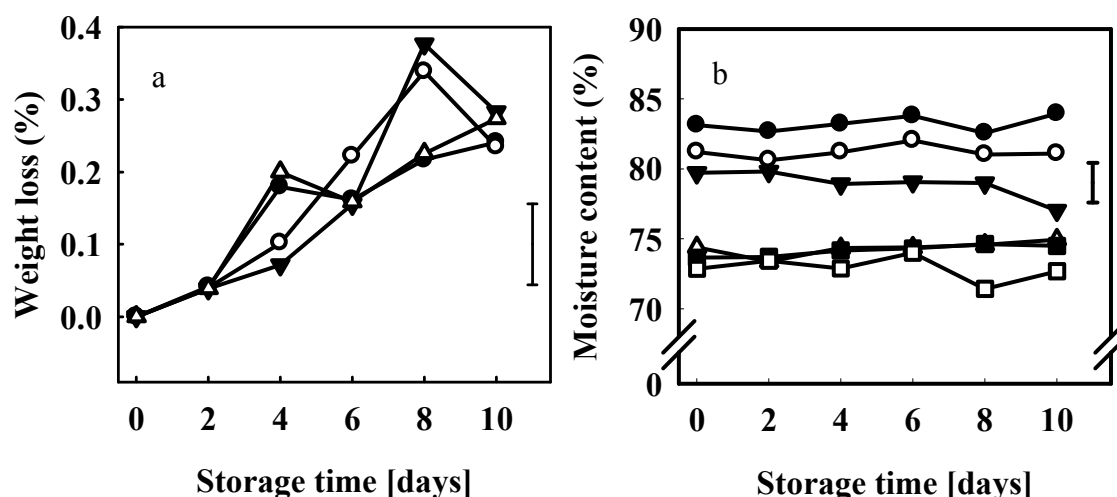


**Figure 6.7:** Moisture content ( $W = [(FW - DW) / FW] * 100 \%$  (g/g)) derived from the sum of values measured in core (●), kernels (○) and shank (▼) of the *sh2* sweetcorn cv. 6800 stored for 10 days at 5°C. The bar indicates the l.s.d. of combined type of tissue and storage time effect.

### 6.3.2.2 Experiment 5

Results from weight loss % in the sweetcorn cobs examined in Experiment 5 (Figure 6.8a) indicated that weight loss increased gradually over time, partly due to dehydration of cobs. This might also be to some extent explained by respiration as both the highest increases of CO<sub>2</sub> and weight loss occurred between day 4 and 8. Weight loss occurred during storage, yet was not significantly differentiated between cvs. and format (window stripped versus naked) of the cobs. The weight loss is probably a result of water loss due to transpiration from the husks which was not as acute as expected during this period of storage (10 days). This is also clear considering that replacement of this water from the kernels was not induced, as moisture content of kernels was not decreased over time. When moisture content of kernels and core in different location of

the cobs (Figure 6.8b) was examined, it was found that the moisture content of core located at the bottom (base) of cobs axis was higher than at the top. However, no significant difference was observed in the moisture content of kernels located in different locations of the cobs.



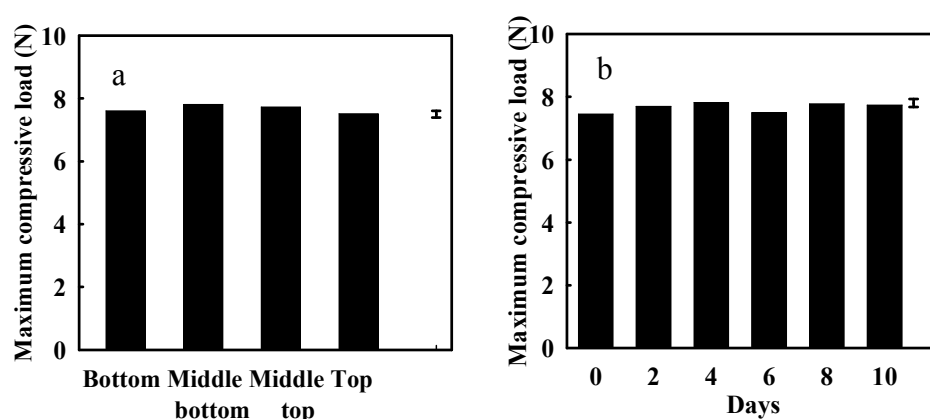
**Figure 6.8 (a):** Weight loss (%) of cv. 7210 in naked (●) and window stripped (○) format and of cv. Fito 206 in naked (▼) and window stripped (Δ) format. The bar indicates the l.s.d. of combined cv. and format. **(b):** moisture content (%) derived from the sum of values measured in bottom (●), middle (○) and top (▼) of the core and bottom (Δ), middle (■) and top (□) of the kernels. The bar indicates the l.s.d. of the interaction of storage time, type of tissue and position of kernels and core in the cob.

### 6.3.3 Maximum compressive load

#### 6.3.3.1 Experiment 4-Results

An important quality attribute of kernels is the texture which can be described through firmness as characterised by maximum compressive load. Results from Experiment 4, showed that there was a main effect of location of kernels in the cobs (Figure 6.9). Kernels located in the middle bottom section of the cobs required significantly higher maximum compressive load for their penetration than kernels

located in the top of the cobs. Furthermore, according to Figure 6.9, at the end of storage period kernels concluded to be significantly firmer than in comparison to the beginning of the storage period, when stored at 5°C. The format of the cobs also had significant effects on kernel firmness. Cobs stored with long shanks were firmer when naked rather than window stripped. However, no significant differences between naked and window stripped cobs were observed when stored with short shanks. The interaction of length of shank with format of the cobs also indicated that naked cobs with long shanks were significantly firmer not only than window stripped with long shanks but also than window stripped cobs with short shanks (Table 6.2).



**Figure 6.9 (a):** Mean maximum compressive load derived from the sum of values measured in kernels located in the bottom, middle bottom, middle top and top section of the cobs, stored at 5°C during 10 days. The bar indicates the l.s.d. of location of kernels in the cobs **(b)** Mean maximum compressive load derived from the sum of values measured in all cobs examined during storage. The bar indicates the l.s.d. of storage time.

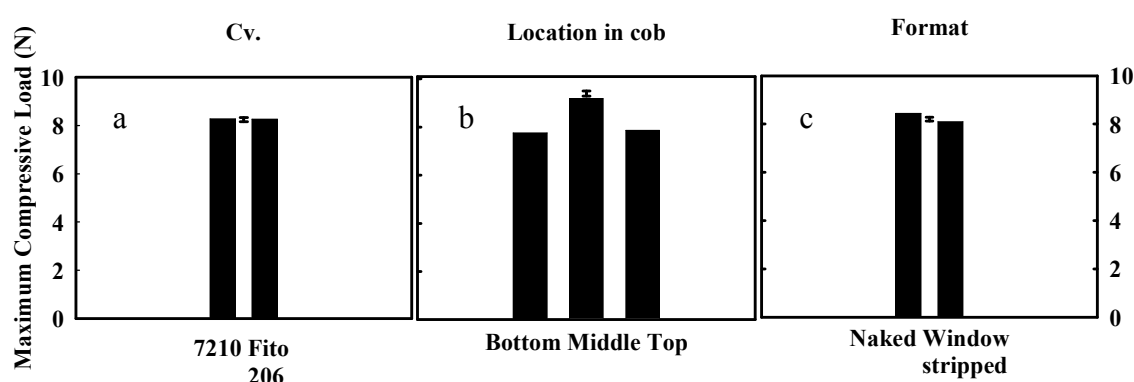
**Table 6.2:** Maximum compressive load values of cobs stored in different format and with different lengths of shanks. The l.s.d. is of combined format and length of shank.

Maximum compressive load (N)		
Format	Length of shank	
	Long	Short
Naked	7.945a	7.736ab
Window stripped	7.430c	7.536bc
l.s.d.:0.2122		

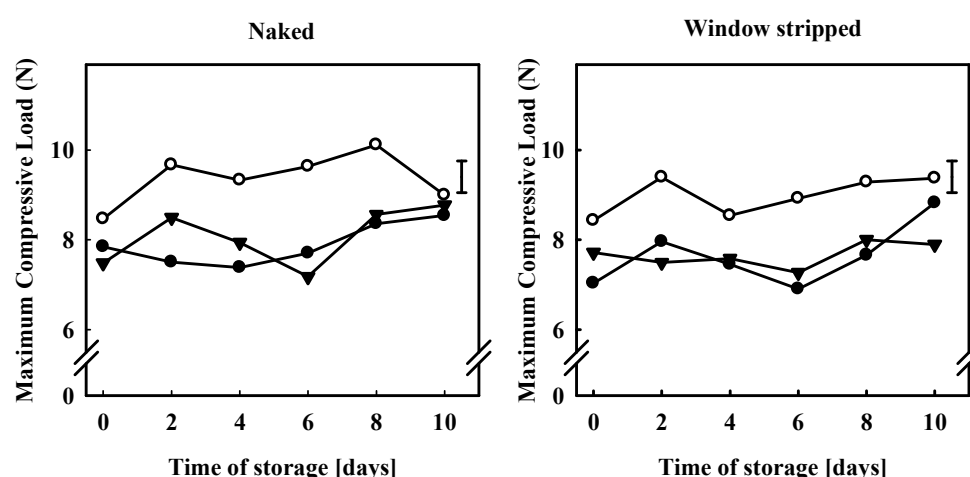
### 6.3.3.2 Experiment 5-Results

Results from Experiment 5, showed that there were not significant differences between mean maximum compressive load values measured in cv. Fito 206 and cv. 7210 (Figure 6.10a). However, format had significant effects on kernel firmness. Naked cobs were significantly firmer than window stripped cobs (Figure 6.10c). On the other hand, it was indicated that kernels located in the middle of the cobs had significantly higher maximum compressive load than kernels located in the bottom and the top of the cobs (Figure 6.10b).

The interaction of storage time, format and location of kernels in the cobs was also significant in terms of firmness. Results showed that generally the maximum compressive load means measured indicated an inconsistent pattern over storage (Figure 6.11).



**Figure 6.10:** Mean maximum compressive load derived from the sum of values measured (a) in cobs of cv. 7210 and of cv. Fito 206, (b) in kernels located in the bottom, middle and top of cobs and (c) in kernels of naked and window stripped cobs stored at 5°C during 10 days. The bars indicate the l.s.d. of cv. (a), cv. (a), location of the kernels in the cobs (b) and format (c).



**Figure 6.11:** Mean maximum compressive load derived from the sum of values measured kernels located in the bottom (●) middle (○) top (▼) of naked cobs and window stripped cobs stored at 5°C during 10 days. The bar indicates the l.s.d. of combined format, storage time and location of the kernels in the cobs.

### 6.3.3.3 Maximum compressive load - Discussion

A similar experiment to Experiment 4, was conducted by Szymanek *et al.* (2003) who used compression, penetration and shearing tests in kernels of *sh2*-corn varieties, other than these used in the current study but without considering storage time, length of shank and format of the cobs as source of variation. The authors stated that no significant difference were apparent between modulus of elasticity (MPa) and shearing energy (J) values measured in kernels located in middle-bottom and middle top positions (Figure 6.1) which is in agreement with the non-significant differences of these two section in the present study.

The variation in firmness of kernels located in different positions of the cobs was in agreement with a previous study (Burton, 1982). In particular, according to Burton as cited by Szymanek (2003), results from penetration tests also indicated that kernels from the central part of cv. Country Gentleman (also known as shoepeg), were less soft than kernels located in the tip and the base of the cobs. A possible explanation given for these results was that kernels were probably in different ripening stages. However, as

was previously mentioned there is a lack of information about changes in texture over storage time.

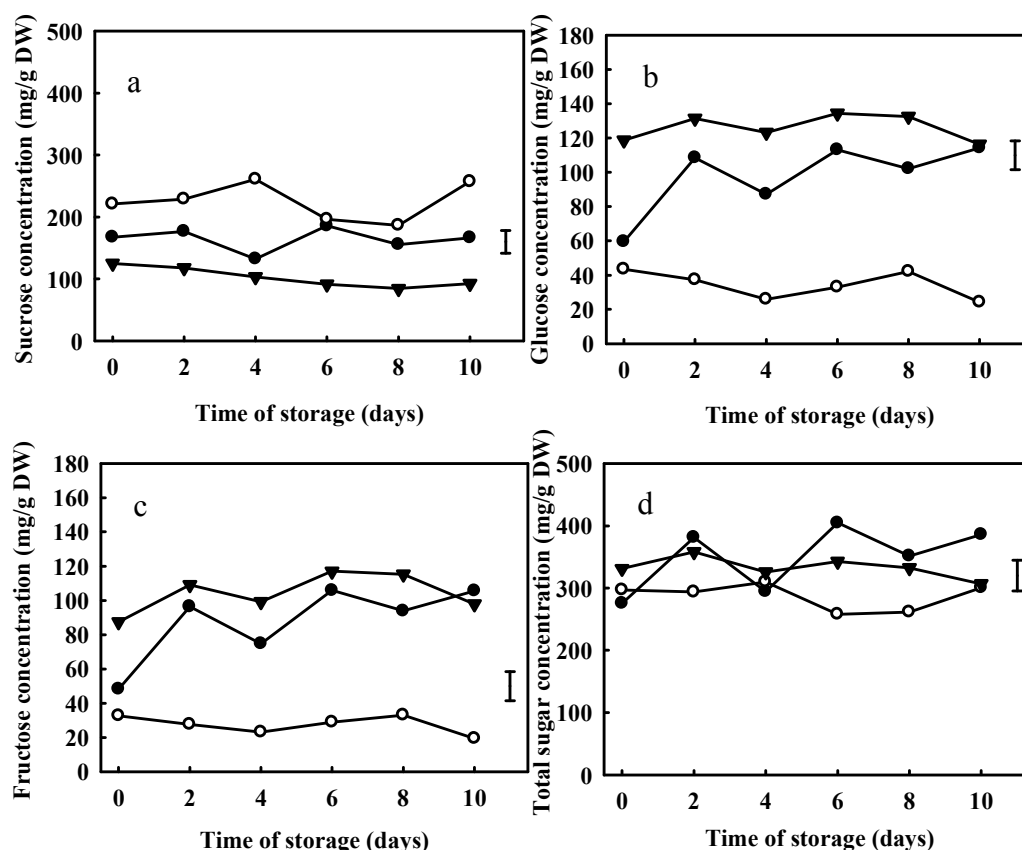
Genotype as source of variation in kernel texture was not apparent in this study. Last but not least, the inconsistent pattern of maximum compressive load means over storage (Figure 6.11) suggests that a statement for a specific trend of firmness over storage would be inappropriate.

### **6.3.4 Sugars**

#### **6.3.4.1 Experiment 4**

Results derived from sugars measurements in samples of Experiment 4, indicated that sugar content of core, as a sum of sucrose, glucose and fructose on dry weight basis, was 1.22-fold higher than that measured in kernels during 10 days of storage while sugar content of shank was 1.16-fold higher than kernels. In more detail, sucrose content in kernels was 1.37-fold greater than in core and 2.2-fold greater than in shank. Total sugars were higher on a dry weight basis in core and shank than kernels as sum of all the days of the experiment. Total sugars concentration of kernels, was significantly decreased at day 6 and 8 on dry weight basis, while total sugars concentration of core was increased the same period (Figure 6.12d). Distribution of sugars on a dry weight basis, (kernels [sucrose 78.49%, glucose 11.96% and fructose 9.55%]; core [sucrose 47.03%, glucose 27.92% and fructose 25.05%]; shank [sucrose 30.77%, glucose 37.89% and fructose 31.34%]), was significantly different between tissues (Figure 6.12).

The length of shank did not affect significantly total sugar content (see Appendix B). According to Table 6.3, the format of the cobs affected significantly total sugar content of core only. Actually, there was evidence that sugar content of core of window stripped cobs was higher than of naked ones.



**Figure 6.12:** Mean concentration (mg/g DW) of sucrose (a), glucose (b), fructose (c) and total sugars (d) on dry weight basis, derived from the sum of values measured in core (●), kernels (○) and shank (▼) of the *sh2* sweetcorn cultivar 6800 stored for 10 days at 5°C (Exp. 4). The bars indicate the l.s.d. of combined type of tissue and storage time effect.

**Table 6.3:** Mean concentration (mg/g DW) of total sugars, derived from the sum of values measured in core, kernels and shank. The l.s.d. is of combined format and tissue type.

Total sugars (mg/g DW)			
Format	Tissue type		
	Core	kernels	shank
Naked	328.1b	287c	341.2b
Window stripped	369.7a	286.7c	324.4b
l.s.d.: 28.04			

In a previous study it was found that the first days after silking, the concentration of glucose and fructose in corn cob of *Zea mays* L. varieties was higher than concentration of sucrose. However, after day 18, sucrose was found in higher concentrations (Bemiller and Hoffman, 1972). Considering that usually harvest period is 18-22 days after silking, the results of this study are in agreement with the current work. Sucrose was also the predominant sugar of samples taken from the shank of the cobs tested, in agreement with previous study which had been conducted using sugary (*su*), sugary enhancer (*su se*), and starchy (*Su*) cultivars (Shaw and Dickinson, 1984). Sucrose in kernels reached peak concentration at day 4, while the same day sucrose concentration of core was at its lowest point. Results derived from mean concentrations of sucrose in shank, showed a general propensity to decline gradually (Figure 6.12a). Lowest means of glucose concentration noticed at day 0 for core and 10 for kernels; and highest means at day 10 for core and 0 for kernels (Figure 6.12b). Krull and Inglett (1980) had stated that glucose content in the core of corn was almost 40% of total sample weight. However, authors did not state the variety used for examination. Furthermore, they did not examine concentrations of sucrose and fructose, or report on temporal changes on sugar content. Thus, and considering that sucrose and fructose also exist, it can be said that the available biomass for production of sugars in fermentation procedures might have even greater potential for the production of energy coming from these by-products. The potential production of energy may also be increased on days when total sugars content is higher, which means the first days of storage rather than the last ones. Bemiller and Hoffmann (1972) had explained decreases in concentration of sugars in stalks and corncobs by increase of cell death. Authors stated that decrease of sugar concentration also meant synthesis of cell components including cell walls. No link was found between sugar concentration of corncob and other tissues in the plant when was studied by Bemiller and Hoffmann (1972). Yet, authors did not reject the hypothesis of a potential link between sugar content of tissues. The current study indicated that there was no correlation between sugar levels in different tissues during postharvest life of sweetcorn [Correlation coefficients: core-shank (-0.1460), core-kernels (-0.026, kernels-shank (0.023)], as the correlation would be significant if the correlation coefficient was greater than 0.1681 or lower than -0.1681. The absence of such correlation probably relies on the fact that changes in the sugar content of kernels



are not a reflection of changes in sugar content of core and shank. Actually, these changes are probably a result of senescence that might impede the cell to cell transportation of sugars. However, another study showed an interdependence of sugar concentrations of various tissues at several stages of development (Russo *et al.*, 2004)

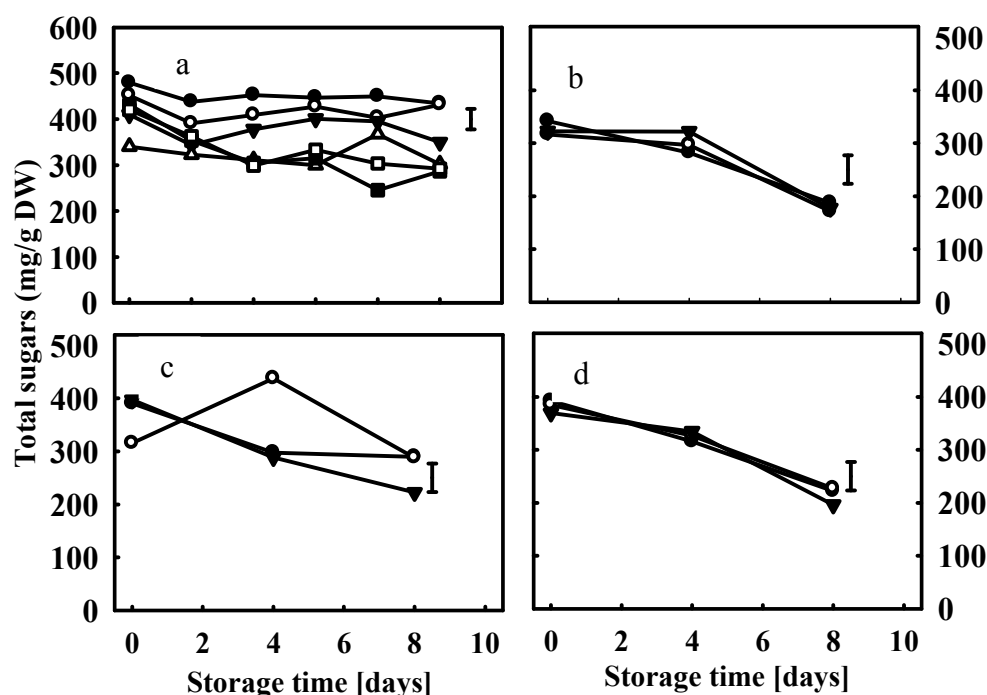
Results regarding the spatial profile of the cobs of cv. 6800 tested at day 0, 4 and 8 of the experiment, showed that sugar content of kernels was significantly lower than that found in the shank and core. Thus, the range of utilities of the non-edible parts of the cobs can potentially be better defined. No significant difference was observed between kernels located in different parts of the cobs and between different parts of the shank.

Hence, sugar content of one type of tissue cannot be used as a rapid indicator of the sugar content of another type of the tissues examined. However, chemical components related to sugar production and/or reduction - such as starch - were not studied. Thus, a relationship between tissue types cannot be discounted due to lack of data available. Results from the part of Experiment 4 which was carried out as precursor of Experiment 5 and referred to spatial distribution of sugars to each type of tissue examined, are presented in next Section.

#### **6.3.4.2 Experiment 5**

Results derived from Experiment 5, were in agreement with Experiment 4, and showed that sugar concentration in the core were higher than in kernels. It was indicated that core located in the bottom of the cob had significantly higher sugar concentration than in the top. Generally, results showed that sugar concentration between different locations in cobs was not significantly differentiated during storage time (Figure 6.13). However, sugar content of kernels taken from the bottom of the cobs was lower in the middle and top of the cobs at day 0 of the experiment, while increased until day 8. At day 8 of the experiment sugar content of kernels located in the bottom of the cobs was higher than in kernels located in middle and top (Figure 6.13a). In all cases, an increase of sugars may reflect sugar accumulation which in turn may be a result of the incapability of plant to utilise them. However, total sugars of the core samples located in the middle part of the cobs were significantly higher than those located in the bottom

and the top. In all cases apart from ‘middle-core’ sugar content of all tissues was significantly decreased over time (Figure 6.13 b, c & d). The quality of cobs examined decreased over storage, as quality of sweetcorn is positively related to sugar content. On the other hand, there was no evidence that sugar content of kernels was affected by their location in the cobs.

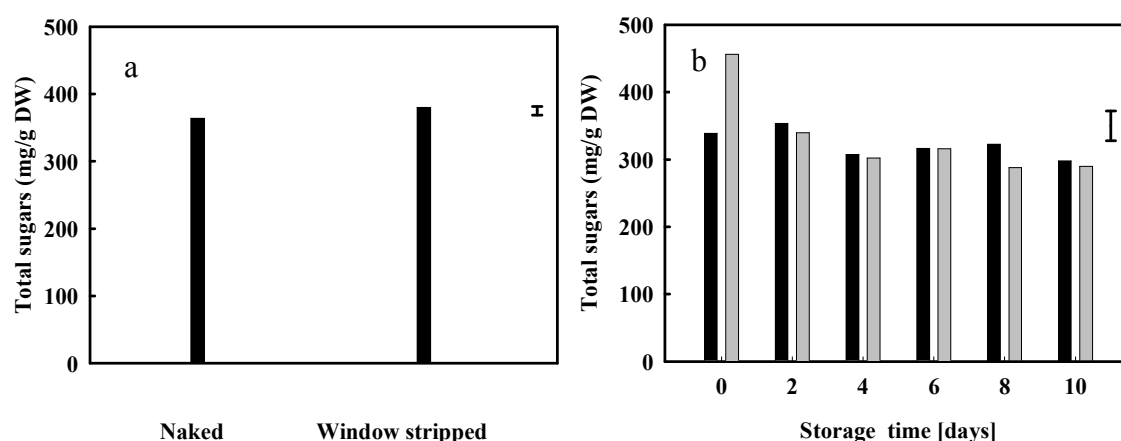


**Figure 6.13 (a):** Mean total sugars dry weight (DW) derived from the sum of values measured in core located in the bottom (●) middle (○) top (▼) and in bottom (Δ), middle (■) and top (□) of kernels of combined cobs of cvs. 7210 and Fito 206 stored at 5°C during 10 days, **(b, c & d):** Mean total sugars dry weight (DW) derived from the sum of values measured in samples located in the bottom (●), middle (○) and top (▼) of kernels, core and shank respectively, as measured from cv. 6800 stored at 5°C during 10 days. The bar indicates the l.s.d. of combined tissue, location of samples in the cobs, and storage time.

Furthermore, total sugar concentration as a sum of total sugar content of core and kernels was significantly different between naked and window stripped cobs. In

particular, total sugar content of window stripped cobs was higher than that of naked cobs (Figure 6.14a). The interaction of tissue type and format did not have any significant effects on total sugar content (see Appendix B). Therefore, it cannot be determined whether format affected total sugar content of kernels or of the core of the cobs examined.

Kernels of cv. 7210 had significantly higher sugar content than kernels of cv. Fito 206 only on the first day of the experiment, which also indicates the rapid decrease of total sugar content in the first two days of storage. In contrast, cv. Fito 206 retained its sugar content during the storage period. The decrease of sugar content of cv. 7210, might be explained by faulty precooling after harvest. On the other hand, differences in sugar content during this time may also be result of genetic variation between the two cvs.



**Figure 6.14:** (a) Mean total sugars dry weight (DW) derived from the sum of values measured in core and kernels of naked and window stripped cobs and (b) of kernels of cv. 7210 (■) and Fito 206 (■). The bar indicates the l.s.d. of format and of combined tissue type, storage time and cv., respectively.

## 6.4 Conclusions

The current study attempted to contribute to a better understanding of texture-related characteristics of naked and window stripped sweetcorn cobs and to create a

more complete spatial profiling of taste-related characteristics of sweetcorn. The null hypothesis of Experiment 4 was confirmed as there was not any interrelationship between sugar and moisture content of kernels, core and shank. The null hypothesis of Experiment 5 was also confirmed as there was no evidence of any interrelationship between texture and sugar and moisture content of bottom, middle and top section of *sh2*-sweetcorn cobs and shank.

The objectives of the study were reached: (1) the sugar profile of kernels, core and shank was determined suggesting that sugar content of core and shank was higher than in kernels during 10 days of storage. Distribution of sugars was statistically differentiated between the different types of tissue. This said, when considering the results on a dry weight basis, temporal changes in the sugar profile through storage were strongly dependent on tissue type and storage duration. (2) However, changes which occurred in the core of corn cobs and shanks were not generally correlated with changes in kernels. Thus, it is suggested that (3) the changes in the non-edible tissues examined, cannot be used to monitor or to predict changes in sugar concentration of kernels during storage; unlike during development of corn. (4) It was also shown that the length of shanks and the presence of husk can be considered as a source of variation in chemical and textural attributes of supersweet sweetcorn. In particular, in terms of texture, naked cobs were generally firmer than the window stripped ones and occasionally cobs with long shank firmer than cobs with short shank. Kernels located in the middle of the cobs, which is also the part used in the markets of cobbettes, were always firmer than kernels located in the bottom and the top.

## 7. CHAPTER SEVEN

### **Physiological and biochemical changes in sweetcorn cv. 7210 under different cooking conditions**

#### **7.1 Introduction**

Supersweet sweetcorn is an important crop in the fresh market of vegetables and is mainly consumed when cooked. Physiological and biochemical alterations caused by microwave cooking for different periods of time and the interaction with postharvest storage time have not been fully defined. The aim of this work was to elucidate changes in the biochemistry and physiology of cv. 7210 held at 5°C for 10 days under different cooking conditions.

The physiological and biochemical profile of sweetcorn has been studied in detail. In particular, carbohydrate and total phenolic content of sweetcorn have been points of interest for several researchers, since these compounds provide important information about consumer preference and potential antioxidant properties, respectively. Starch, the major hydrocarbon component of sweetcorn is produced in very low amounts in *sh2* sweetcorn cobs. Jennings and McCombs (1969) reported that sucrose accumulation in *sh2* genotypes might be related to starch degradation. Later, it was shown that high sugar content in cvs. with this recessive gene, is maintained better than in standard genotypes, due to prohibited transformation of sugars to starch. The conversion of sugars to starch is also related to the decline of moisture content (Douglass *et al.*, 1993).

Phenolic compounds (including flavonoids, tocopherols and phenolic acids) are natural antioxidant compounds. Phenolic compounds exist in free and bound forms and can have a role in resistance of kernels to pests however in recent times interest has centred around their health-promoting properties. Among the phenolic compounds identified in sweetcorn, ferulic acid is of special interest due to the antioxidant properties it has. Furthermore, the relationship between total antioxidant capacity and

total phenolic content has been assessed in several studies (Sen *et al.*, 1994; Kahkonen *et al.*, 1999; Dewanto *et al.*, 2002; Sun *et al.*, 2002;).

Whilst the biochemical profile of raw sweetcorn has been studied extensively, the effects of cooking on biochemical composition have only been investigated to some extent (Hart and Scott, 1995; Boyes *et al.*, 1997; Riad and Brecht, 2003; Riad *et al.*, 2003). Furthermore, the effects of the interaction of storage time and cooking time on firmness, and sugar and phenolic content of sweetcorn have not been thoroughly elucidated.

Thus, this chapter refers to Experiment 6 that aimed to elucidate the biochemical and texture-related changes occurring during 10 days of storage of sweetcorn cobs held at 5°C, and then the interaction between these changes and variable cooking times in the microwave. The null hypothesis of this experiment was that the interaction of storage period and cooking time does not affect the quality of *sh2*-sweetcorn and the alternate hypothesis was that quality of *sh2*-sweetcorn is affected by storage period and cooking time. The objectives of the work were: (1) to elucidate the nutritional loss due to cooking in the microwave for different durations, (2) to establish the optimum cooking time that should not be exceeded, in order to avoid significant sugar loss, (3) to compare nutritional values of fresh and cooked sweetcorn.

## 7.2 Materials and Methods

The yellow variety 7210 (n=120) was used for the current study. Cobs were harvested on 17<sup>th</sup> August 2009 from the area of Chichester, West Sussex, UK and packed, as per commercial practice, after removal of husks. Cobs were then transferred within 3 hours to Cranfield University as described in Section 5.3, where they were stored immediately in a controlled temperature room set up at 5°C. The experiment lasted 10 days and day 0 was considered the day after the cobs arrived (18<sup>th</sup> August, 2009). All cobs were weighed at the first day of storage and at each outturn in order to calculate weight loss. For fresh cobs (i.e. not cooked), kernels were also tested for their colour properties [n=120 (cobs) x 3 (replicates) = 360; (see Section 3.5)]. Texture measurements [12 penetrations tests per cob (see Section 3.6.2)] and sample preparation for subsequent analyses was performed every second day of the experiment [days: 0, 2,

4, 6, 8 and 10; (n=20/day)]. At each outturn, four cobs were processed as fresh cobs and the remaining sixteen cooked in a Panasonic, NN-CD757W microwave [cobs cooked for 2.5 min (n=4); 5 min (n=4); 7.5 min (n=4) and 10 min (n=4)]. The protocol used to cook the sweetcorn cobs was based on a home cooking protocol as recommended on sweetcorn packaging used by Tesco Stores: for each cooking procedure, two cobs were placed in a microwavable bowl with 30 mL of water (water temperature = 18°C). The microwavable bowl was covered with a lid and the microwave was set up to work on full power (Category E). The stand time in the microwave before any penetration test was 1 min. Only one of the two cobs was chosen randomly for further analysis. After penetration tests, cobs were left to cool down to approximately 80°C and then cut, snap-frozen and stored at -40°C for subsequent measurements of sugars, starch, total phenolics and moisture content as described in Chapter 3.

## 7.3 Results and discussion

### 7.3.1 Colour properties of kernels and CO<sub>2</sub> concentration in packaging

All colour parameters were affected by storage duration. In particular, hue angle of fresh cobs was significantly lower at the end of the experiment when compared to the first day. However, until day 8 no significant differences were observed, with the yellow colour generally being maintained. Mean values of chroma and lightness also decreased over time, indicating that in the first days of storage, sweetcorn kernels had a lighter colour (Table 7.1). Riad *et al.*, (2003) also found that hue angle of fresh-cut kernels of cv. Prime Time, after 10 days of CA storage (2% O<sub>2</sub>-10% CO<sub>2</sub>) at 5 °C decreased, however chroma values in contrast to the results of the current study increased probably due to variation between cvs. The decrease of colour properties might be a result of storage temperature, as Kader (2004) had suggested that inappropriate storage temperature may lead to greater tissue discolouration. On the other hand, the concentration of respiration gas CO<sub>2</sub> (%) was significantly affected by storage time. In particular, respiration rate and therefore CO<sub>2</sub> concentration in packages

increased during storage and reached the highest concentration at day 10 of the experiment.

**Table 7.1:** Effect of storage period on colour parameters and CO<sub>2</sub> (%) concentration of cv. 7210 stored at 5°C.

Day	Chroma (C*)	Hue angle (h°)	Lightness (L*)	CO <sub>2</sub> (%)
0	48.78a	86.5a	74.99a	6.61c
2	48.29a	86.24ab	74.94a	8.75bc
4	46.43b	86.44a	72.49b	10.88ab
6	45.49bc	86.29ab	72.03b	10.58ab
8	44.96c	86.24ab	73.33b	10.84ab
10	42.02c	83.43b	69.99c	11.46a
l.s.d:	1.301	2.922	1.508	2.417

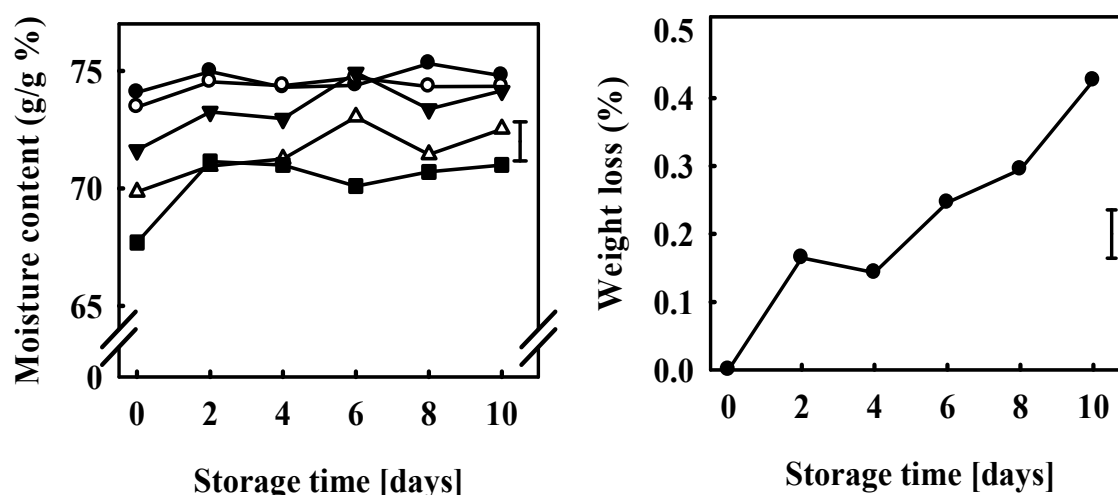
Correlations of colour parameters and CO<sub>2</sub> (%) are shown in Table 7.3. For all correlation coefficients reported in Table 7.3 the level of correlation was considered significant when the calculated correlation coefficients were lower than 0.4132/0.5526 and 0.7282 or greater than 0.4132/0.5526 and 0.7282 for a significance level of 0.05/0.01 or 0.001 respectively). Significant correlation coefficients are marked with stars depending on the strength of correlation as explained in the Table.

### 7.3.2 Moisture content and weight loss

Kernels cooked for 2.5 min were not significantly different in their moisture content compared to raw cobs. The longer the cooking time, the greater the decrease in moisture content (correlation coefficient as derived from all cobs examined during storage: -0.757). Generally, moisture content did not decrease significantly over time for fresh kernels and kernels cooked for 5 min or less. On the other hand, weight loss which according to Türk *et al.* (2001) is mainly observed as water loss, was elevated over time (Figure 7.1) probably due to respiration-release of CO<sub>2</sub> (Table 7.2). Furthermore, increased weight loss is linked to the natural process of sweetcorn deterioration occurring over storage time (Aharoni *et al.*, 1996). Riad (2004) did not observe denting



incidence and lowered appearance quality when weight loss ranged from 0.05-0.22%. Increased relative humidity was considered as the reason for the insignificant weight loss in the study mentioned. Results from the current study indicated that at the end of the storage period, the rate weight loss was lower than at the beginning in agreement with the observations of Türk *et al.* (2001) even though the authors examined weight loss of cobs stored for 7 weeks at 0°C.

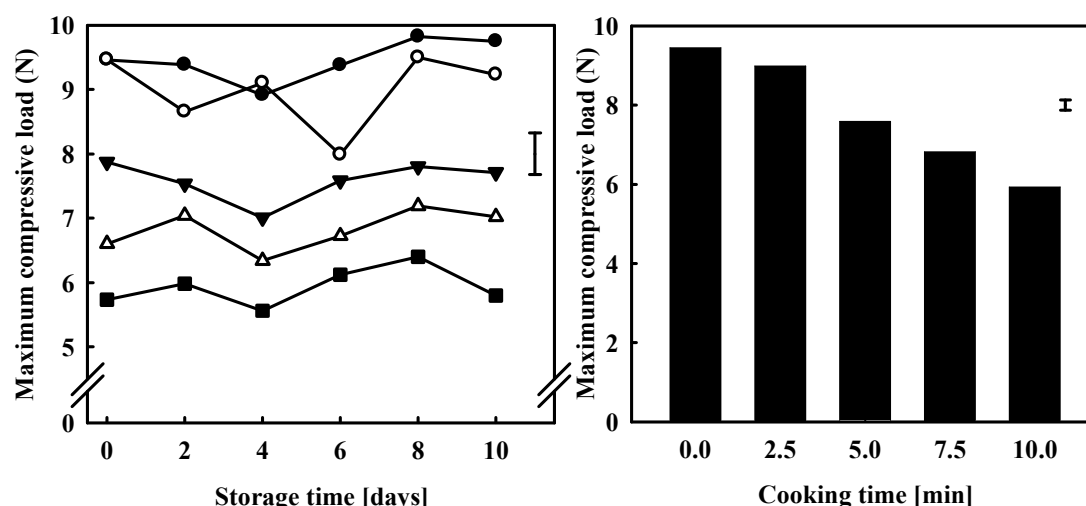


**Figure 7.1:** Moisture content of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (Δ) and 10 min (■) stored at 5°C during 10 days of storage and weight loss (%) during storage. The bars indicate the l.s.d. of combined cooking and storage time and the l.s.d. of storage time respectively.

### 7.3.3 Firmness

Cooking time significantly affected maximum compressive load. In particular, increased cooking time led to decreased maximum compressive load values. Thus, firmness was higher for fresh cobs in comparison to cobs cooked for 2.5; 5; 7.5 and 10 min. Hence, firmness of cobs examined, decreased with increased cooking time (Figure 7.2). The negative relationship of firmness and cooking time is in agreement with Bourne (1982) who stated that loss in firmness was negatively correlated with temperature. While the interaction of cooking time and storage time influence the

maximum compressive load measured in the cobs examined, this influence was not always significant. For instance, on most days, firmness of fresh cobs was not significantly different from firmness of cobs cooked for 2.5 min. However, in the plot of Figure 7.2 that displays only the effects of cooking time, it is indicated that all categories of cooking time examined, had significantly different firmness between them.



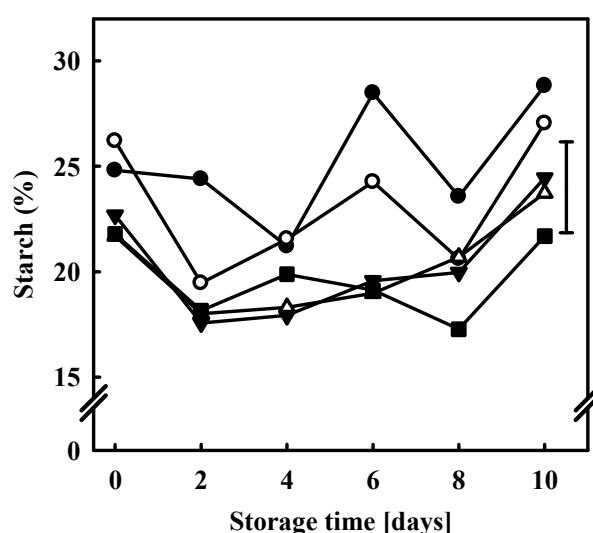
**Figure 7.2:** Mean maximum compressive load values of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (Δ) and 10 min (■) stored at 5°C during 10 days and mean maximum compressive load values of cv. 7210 in fresh and cooked samples for 2.5 min, 5 min, 7.5 min and 10 min as derived from the values derived from all the 10 days of storage. The bars indicate the l.s.d. of combined cooking and storage time and of cooking time respectively.

### 7.3.4 Starch

Starch content of the cobs fluctuated over storage (Figure 7.3). In addition to moisture content which is associated with starch (Tracy, 1997), starch content also did not change significantly during storage. Türk *et al.* (2001) found fluctuation in starch content during storage but only explained the increased starch content as a result of conversion of sugars to starch. Correlations derived from the present study are shown in Table 7.3 for raw sweetcorn cobs and in Table 7.4 for cooked cobs. For all correlation

coefficients reported in Table 7.4 the correlation level was considered significant when the calculated correlation coefficients were lower than -0.201/-0.262 and -0.331 or greater than 0.201/0.262 and 0.331 for a significance level of 0.05/0.01 or 0.001 respectively. Significant correlation coefficients have marked with stars depending on the strength of correlation as explained in the Table. Correlations shown are relatively poor and are discussed where necessary.

One of the favourable characteristics of *sh2* sweetcorn is the slow conversion of sugars to starch (Jennings and McCobs, 1969) and this was apparent in the present results as no significant difference was observed in starch content of the cobs examined between day 0 and day 10 (Figure 7.3).



**Figure 7.3:** Mean starch content (%) values of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (△) and 10 min (■) stored at 5°C during 10 days. The bar indicates the l.s.d. of cooking and storage time.

Furthermore, total starch content declined with increased cooking time (Table 7.2). Besides, it is well known that upon cooking, starch is hydrolised to dextrins and therefore is decreased (Davis, 1995). Fresh cobs had significantly higher starch than cooked cobs and cobs cooked for 2.5 min had higher starch content than cobs cooked for longer time. According to Table 7.2, when cobs were cooked for longer than 5 min, starch content was not affected significantly.

**Table 7.2:** Total starch (%) as affected by cooking time. L.s.d. indicates the least significant difference of cooking time.

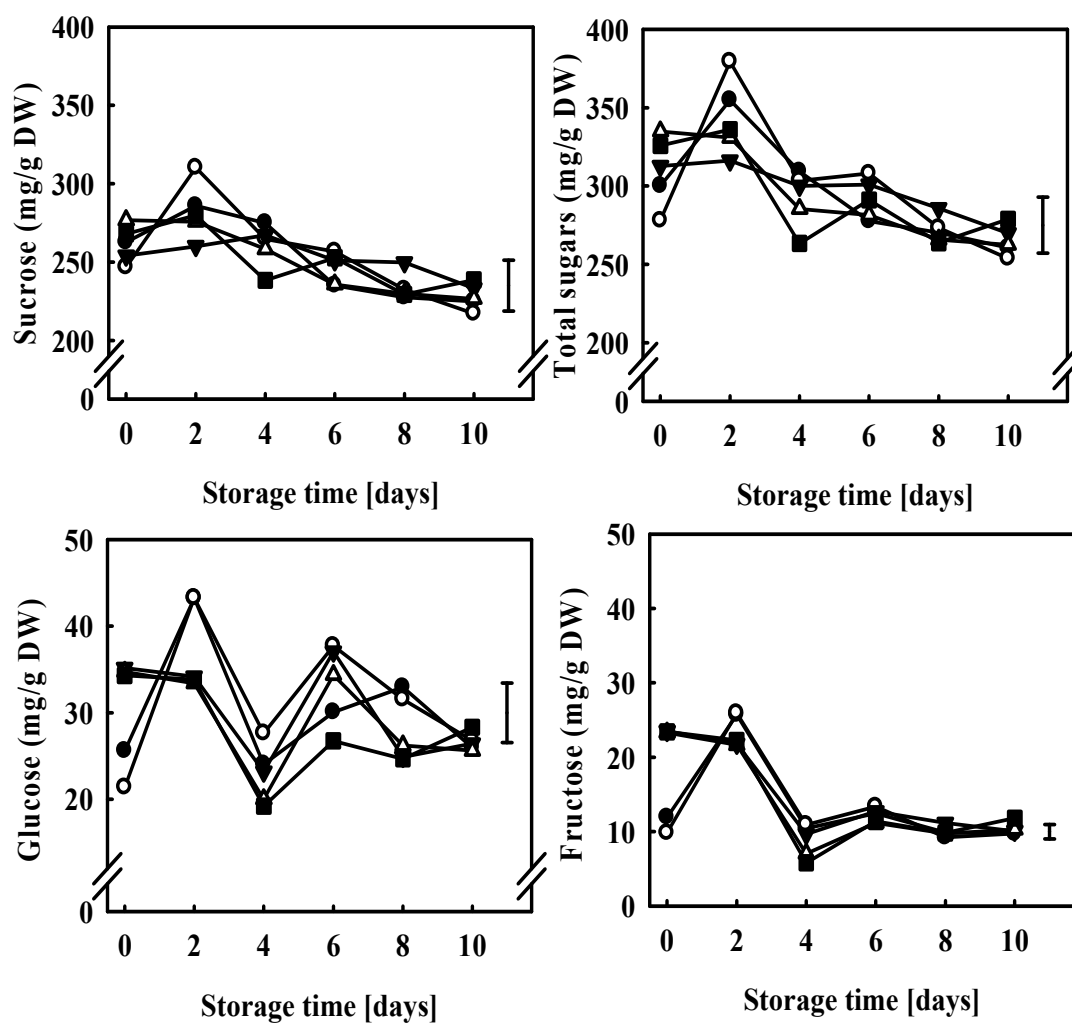
Total starch (%)				
<u>Cooking time [min]</u>				
0	2.5	5	7.5	10
25.21a	23.19b	20.36c	20.23c	19.65c
L.s.d.: 1.761				

### 7.3.5 Sugars

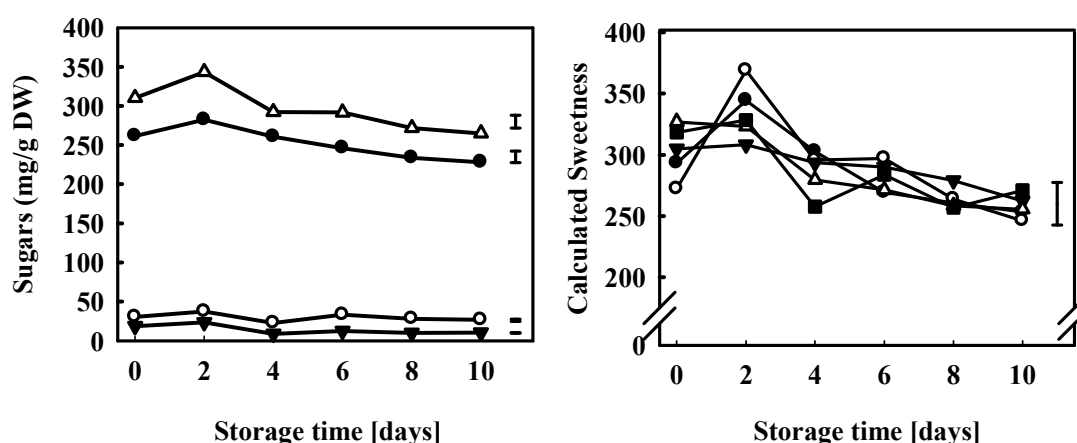
Storage time had a negative effect on sucrose content. Generally, at each day of the experiment no significant differences in sucrose content were observed due to cooking, with just a few exemptions such as the very high sucrose concentration on day 2 of the experiment (Figure 7.4).

Occasionally, glucose content increased or decreased significantly over time. For example, fresh sweetcorn kernels had significantly higher glucose concentration than cooked kernels at day 2 (Figure 7.4). A previous study had suggested that sucrose and fructose content were negatively correlated, while glucose and sucrose content were not (Wong *et al.*, 1994).

The second half of the storage period, total sugar content and sweetness between fresh and cooked kernels, were not significantly differentiated. Total sugar content of cobs cooked for 7.5 and 10 min had declined significantly during storage (Figure 7.4 and 7.5). Riad *et al.* (2003) also stated a significant reduction in total soluble sugars during storage and a decrease with cooking. However, the reduction of sugars in cooking, as authors speculated, was a result of dilution from the cut-surfaces. Moreover, it has been reported that microwave cooking has better retention of nutrients when compared to other methods of cooking (Schnepf and Driskell, 1994). When observing results for sucrose concentration and sweetness it was obvious that Figure 7.4 and 7.5 are quite similar, in agreement with Reyes *et al.* (1982), according to whom; sweetness is highly related to kernel sucrose content. These results are in agreement with the relevant correlations (Table 7.3 and 7.4).



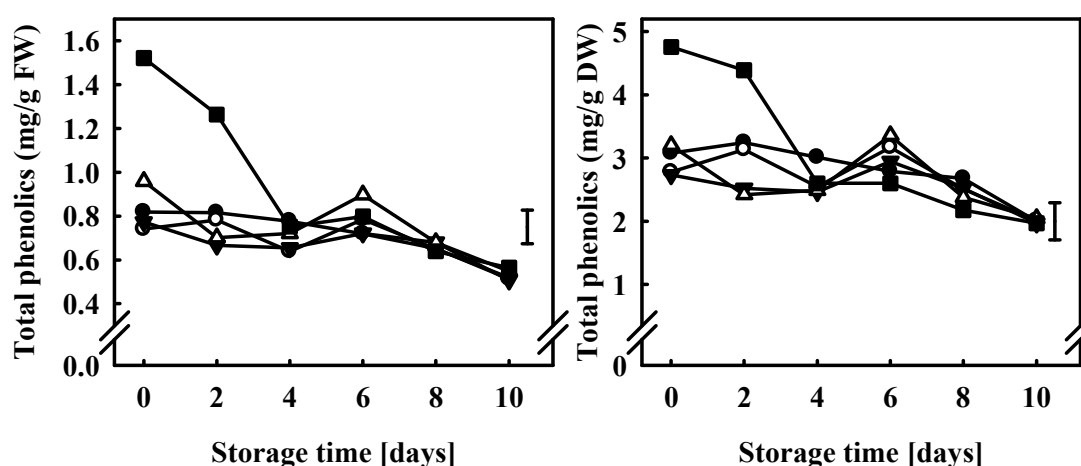
**Figure 7.4:** Mean sugar concentrations values (mg/g DW) of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (Δ) and 10 min (■) stored at 5°C during 10 days. The bars indicate the l.s.d. of combined cooking and storage time.



**Figure 7.5:** Mean sucrose (●), glucose (○), fructose (▼) and total sugars (Δ) concentrations as measured in fresh and cooked samples [for 2.5 min, 5 min, 7.5 min and 10 min (■)] obtained from sweetcorn kernels of cv. 7210 stored at 5°C, during 10 days. Mean sweetness values of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (Δ) and 10 min (■) stored at 5°C during 10 days. The bars indicate the l.s.d. of cooking and of combined cooking and storage time respectively.

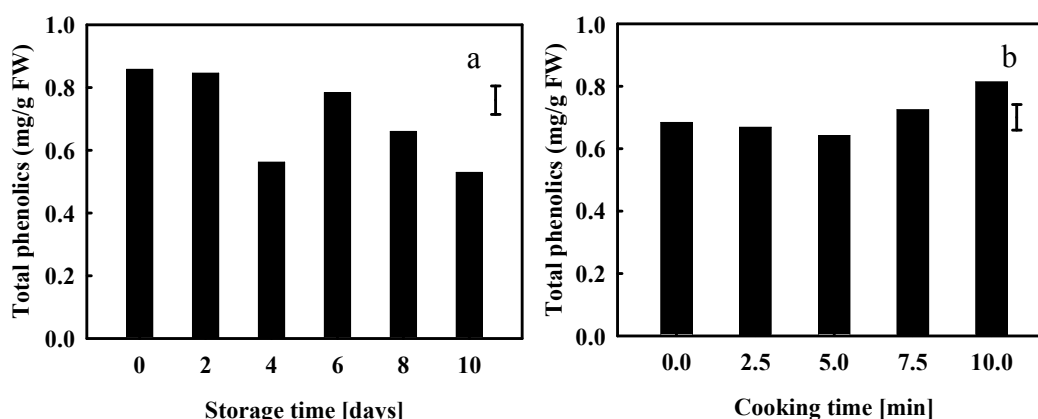
### 7.3.6 Total phenolics

Total phenolic content of all cobs tested decreased during storage. Furthermore, total phenolic content was significantly greater in kernels from sweetcorn cobs of cv. 7210 cooked for 10 min at day 0 and 2 of the storage period, on both a fresh and dry weight basis (Figure 7.6). Riad *et al.* (2003) reported that total soluble phenolics content of sweetcorn kernels stored at 5°C, decreased with cooking but did not change significantly during storage. However, in the study of Riad *et al.* (2003), the kernels examined were stored as fresh-cut kernels. It has also been stated that bound phenolic content decrease and free phenolic content increase with longer heating time. In the current study, total phenolic content was estimated as a combination of free and bound phenolic content as samples had been freeze-dried and it has been speculated that this process releases more bound phenolic compounds (Dewanto *et al.*, 2002).



**Figure 7.6:** Mean total phenolic content values (mg/g DW and FW) of cv. 7210 in fresh (●) and cooked samples for 2.5 min (○), 5 min (▼), 7.5 min (Δ) and 10 min (■) stored at 5°C during 10 days of storage. The bars indicate the l.s.d. of cooking and storage time.

The statistical analysis indicated that on both dry and fresh weight basis the period of storage and the interaction of storage time and cooking time had significant influence on total phenolic content (see Appendix B). However, on fresh weight basis, there was evidence that cooking time as a unique factor of influence, affect significantly total phenolic content in contrast to dry weight basis. On a fresh weight basis, sweetcorn cobs cooked for 10 min (Figure 7.7b) had greater total phenolic content than fresh cobs and cobs cooked for shorter period. On the other hand at the end of the experiment, total phenolic content was significantly lower than at the beginning (Figure 7.7a). Hence, total phenolic content is adversely affected by storage time.



**Figure 7.7 (a):** Mean total phenolic content values (mg/g FW) of cv. 7210 during storage. The bar indicates the l.s.d. of storage time. **(b)** Mean total phenolic content values (mg/g FW) of cv. 7210 examined as fresh and cooked for 2.5 min, 5 min, 7.5 min and 10 min stored at 5°C during 10 days of storage. The bar indicates the l.s.d. of cooking time.

Results suggest sweetcorn should be consumed in the early days of storage as the potential concentration of health-promoting compounds declines during storage time. There is also an indication that during the first days of storage, cobs microwaved for 10 min have greater antioxidant properties than uncooked and cooked cobs for 5 min. However, the poor correlation between total phenolic content and total sugars that was observed (Table 7.4) in combination with the absence of correlations involving colour, ferulic acid concentration and total antioxidant capacity renders the results inadequate and potentially imprecise for valid conclusions. The interference of reducing sugars in the method used and/or the esterification of phenolic acids to carbohydrates (Sen *et al.*, 1994) after which hardening of cell-wall tissues follows, can probably explain the correlation found between sugars and total phenolic content of cooked cobs (Table 7.4). Thus, the need to clarify the changes and the accuracy of the results derived, lead to the experimental design described in Chapter 8.



**Table 7.3:** Correlation coefficients between quality parameters regarding fresh-uncooked sweetcorn

		Correlation coefficients									
<sup>1</sup> C	1.000***										
CO <sub>2</sub>	-0.406	1.000***									
<sup>2</sup> H	0.348	-0.393	1.000***								
<sup>3</sup> L	0.21	-0.443*	0.204	1.000***							
<sup>4</sup> M.C.L.	0.252	0.207	-0.041	-0.326	1.000***						
<sup>5</sup> S.	0.31	-0.568**	0.233	-0.250	-0.039	1.000***					
<sup>6</sup> T.S.	0.29	-0.559*	0.221	-0.262	-0.063	0.999***	1.000***				
<sup>7</sup> Fr.	0.135	-0.348	-0.102	-0.263	-0.202	0.758***	0.777***	1.000***			
<sup>8</sup> Gl.	-0.164	-0.126	-0.168	-0.324	-0.420	0.514*	0.551**	0.858***	1.000***		
<sup>9</sup> M.C.	-0.473	0.359	-0.214	-0.176	0.101	-0.353	-0.331	0.075	0.268	1.000***	
Starch	-0.001	0.002	-0.229	0.237	-0.198	-0.181	-0.172	0.195	0.174	-0.022	1.000***
Sucrose	0.372	-0.592**	0.332	-0.194	0.063	0.963***	0.952***	0.275	-0.487*	-0.290	1.000***
<sup>10</sup> T.P.	0.148	-0.149	0.256	0.159	-0.015	0.041	0.039	0.117	0.318	0.318	1.000***
<sup>11</sup> W.L.	-0.497*	0.639**	-0.392	-0.226	-0.1	-0.631**	-0.615**	-0.412	-0.083	0.341	-0.66**
C	CO <sub>2</sub>	H	L	M.C.L.	Sucrose.	T.S.	Fr.	Gl.	M.C.	Starch	Sucrose
										T.P.	W.L.

\* Strong correlation at significant level 0.05, \*\* Strong correlation at significant level of 0.01, \*\*\*Strong correlation at significant level of 0.001.

<sup>1</sup>chroma, <sup>2</sup>hue angle, <sup>3</sup>lightness, <sup>4</sup>maximum compressive load, <sup>5</sup>sweetness, <sup>6</sup>total sugars, <sup>7</sup>fructose, <sup>8</sup>glucose, <sup>9</sup>moisture content, <sup>10</sup>total phenolics, <sup>11</sup>weight loss

**Table 7.4:** Correlation coefficients between quality parameters of cooked sweetcorn

Correlation coefficients										
<sup>1</sup> M.C.L.	1	***								
Sweetness	-0.059	1	***							
<sup>2</sup> T.S.	-0.045	0.999	***	1	***					
Fructose	-0.091	0.769	***	0.772	***	1	***			
Glucose	0.117	0.633	***	0.669	***	0.732	***	1	***	
<sup>3</sup> M.C.	0.506	***	-0.061	-0.031	-0.142	0.320	***	1	***	
Starch	0.116	-0.005	-0.011	-0.011	0.061	-0.048	0.032	***	1	***
Sucrose	-0.070	0.960	***	0.952	***	0.578	***	-0.095	-0.016	1
<sup>4</sup> T.P.	-0.265	***	0.403	***	0.401	***	0.330	***	-0.251	***
	M.C.L. <sup>1</sup>	Sweetness	T.S. <sup>2</sup>	Fructose	Glucose	M.C. <sup>3</sup>	Starch	Sucrose	T.P. <sup>4</sup>	

\* Strong correlation at significant level 0.05, \*\* Strong correlation at significant level of 0.01, \*\*\* Strong correlation at significant level of 0.001.

<sup>1</sup>Maximum compressive load, <sup>2</sup>total sugars, <sup>3</sup>moisture content, <sup>4</sup>total phenolics

## 7.4 Conclusions

The loss of chemical compounds such as sugars, starch and phenolic compounds after microwave cooking of sweetcorn cobs held at 5°C during 10 days and the correlation between these compounds was not as significant as expected. The interaction of storage and cooking time appeared to be a significant source of variation in the quality parameters measured and hence the null hypothesis of this experiment was rejected. Starch and firmness were greater in raw sweetcorn while cobs cooked for 10 min had greater total phenolic content which might indicate either greater potential of antioxidants properties or increased cell wall lignin concentration and therefore induced damage in kernels. However, further investigation is required to determine the appropriateness of the total phenolic assay used regarding sweetcorn samples. Furthermore, it is difficult to define a cooking duration that should not be exceeded, as complementary chemical analysis is required for this purpose. Yellowness was retained over time. Yet, the potential relationship between colour properties and carotenoid content were not elucidated. Thus, an experiment that provides further biochemical analysis and also involving more factors of variation is described in the next chapter.

## CHAPTER EIGHT

### **Physiological and biochemical alterations of sweetcorn cv. 6800 as affected by several storage factors and cooking**

#### **8.1 Introduction**

Increased consumer requirements and preferences for certain sweetcorn characteristics led to a well documented physiological and biochemical development of sweetcorn types by means of breeding. However, the alterations in quality attributes which occur in *sh2*-sweetcorn cultivars upon cooking are not as yet fully elucidated and understood. The aim of this study was to elucidate the nutritional changes occurring after cooking sweetcorn cobs of cv. 6800; as affected by format of the cobs (naked vs. window stripped) and temperature (2°C vs. 7°C) over 8 days of storage. The desire to simulate storage practices used in market and at home led to the selection of the storage temperatures applied. In particular, the storage temperatures applied, are common refrigeration and holding temperatures used at suppliers.

Supersweet sweetcorn has high sugar content which is considered as a major quality attribute. However, *sh2*- sweetcorn also contains several antioxidants related to health protection which have not been fully studied. In the last decades research has been conducted to evaluate the concentration and profile of such compounds in sweetcorn adding more quality factors affecting the shelf life of corn. Apart from the total antioxidant activity which is usually analysed to assess the capacity of known and unknown antioxidants a deeper knowledge of antioxidant attributes in sweetcorn can be derived from measurements of total phenolics and individual analysis of carotenoids, ferulic acid and L-ascorbic acid (Tracy *et al.*, 1997; Dewanto *et al.*, 2002; Fanning *et al.*, 2007).

Sugars are the major quality attribute of corn; however antioxidant compounds with potential protective roles in health have lately been promoted. Carotenoids are natural yellow/orange pigments which can aid against degenerative diseases and damage induced by oxidation. (Ribaya-Mercado *et al.*, 2004). Phenolic compounds are

known to be induced under stress conditions and have potentially an important role in health promotion as they have the ability to scavenge free radicals (Atkinson *et al.*, 2005). Among the antioxidants that can prevent damage caused by the exposure of cells to free radicals are ferulic acid and vitamin C (Wheeler *et al.*, 1998; Trombino *et al.*, 2004; Srinivasan *et al.*, 2007; Xiao *et al.*, 2009). Therefore, the identification and quantification of ferulic acid and vitamin C is essential for a better understanding of antioxidant compounds of sweetcorn.

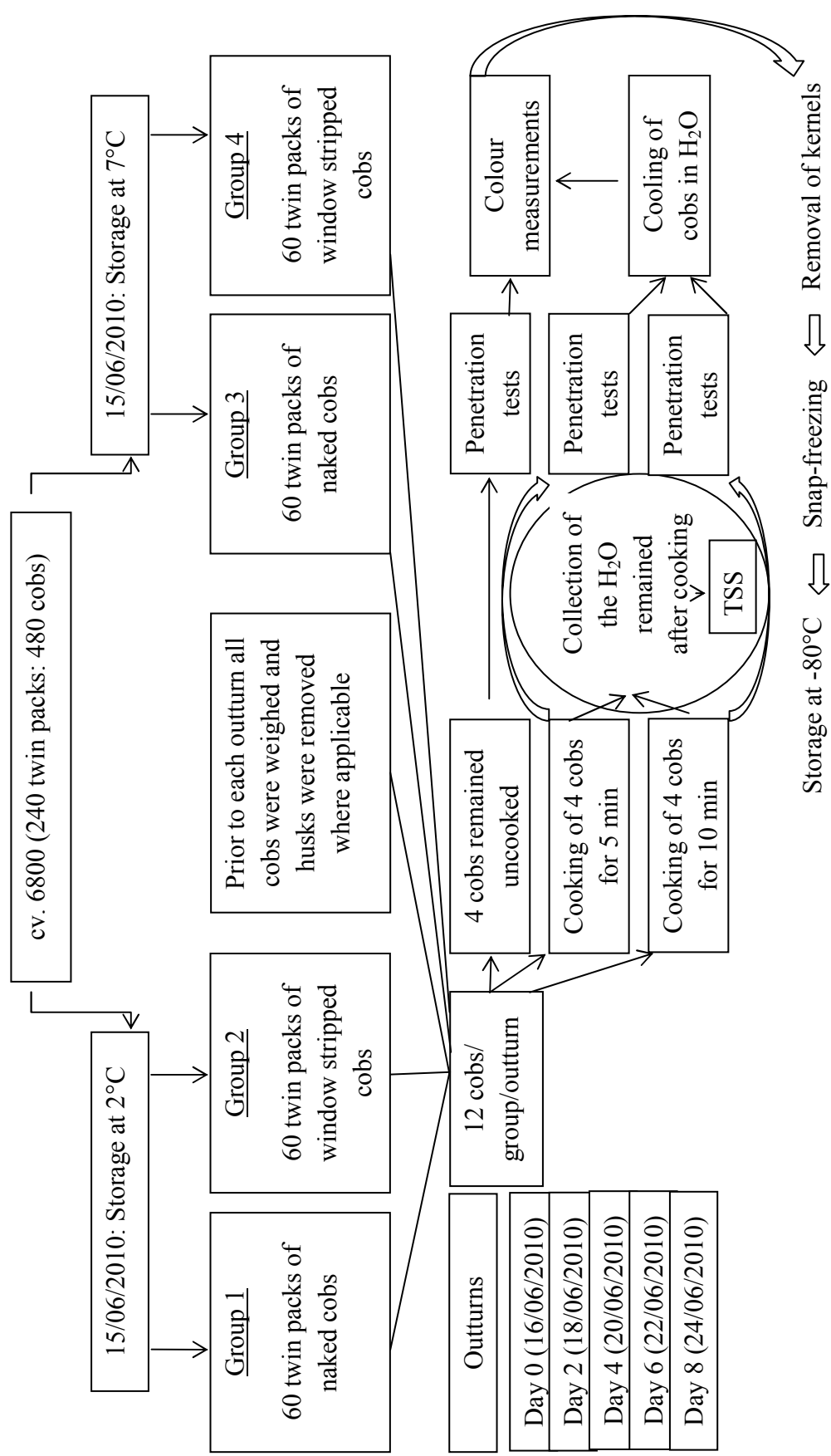
While potential antioxidant compounds and their concentration in corn have been studied, there is a lack of information about the factors that affect their content during postharvest life such as storage temperature and retail format of the cobs. Thus, this chapter aims to create a profile of several quality parameters of sweetcorn focusing on antioxidant compounds, as affected by cooking in the microwave. Evaluation of the effects of format of the cobs (naked vs. window stripped), storage time of two storage temperatures commonly used in the market and at home (2 vs. 7°C) and suggestion of optimum storage conditions is also attempted. Last but not least, it is evaluated whether colour changes can be useful for a rapid evaluation of changes occurring in ferulic acid content.

The null hypothesis of the present study (Experiment 7) was that storage temperature, presence of husks, storage time and cooking time does not affect major quality attributes of sweetcorn (sugars and texture) neither its content of basic antioxidant compounds, and the alternate hypothesis that it does.

## 8.2 Materials and Methods

The yellow sweetcorn variety 6800 (n=480) was used for the current study. Cobs were drilled in Utrera (Spain) on 12<sup>th</sup> of March 2010, harvested and packed in 240 twin packs on the 15<sup>th</sup> June at Barfoots of Botley Ltd (Chichester, West Sussex, UK) and then transferred within 3 hours to Cranfield University as described in Section 5.3. At Cranfield University sweetcorn cobs were stored immediately in controlled temperature rooms set at either 2°C [n=120 twin packs of which: n=60 with naked cobs + n=60 with window stripped cobs] or at 7°C [n=120 twin packs of which: n=60 with naked cobs + n=60 with window stripped cobs]. The experiment lasted 8 days and day 0 was

considered the next day after cobs arrived (16<sup>th</sup> of June, 2010). All cobs were weighed at day 0 and thereafter, as described in Section 6.3. Upon opening the packs, only one of the two cobs from each twin pack was chosen for subsequent measurements and analyses, in order to ensure the randomised design of the experiment. Colour properties were measured in sweetcorn kernels of raw cobs [ $n=80$  (cobs)  $\times$  3 (replicates) = 240]. Colour characteristics in the husks of window stripped cobs were also measured ( $n=120$ ). Colour properties of cooked cobs were measured after cobs were placed in plastic bags and dipped in water until their temperature reached 18°C so that heat-induced damage to the colorimeter would be avoided [ $n=160$  (80+80 cobs cooked for 5 min and 10 min respectively)  $\times$  3 (replicates)=540]. Texture measurements [12 penetrations tests per cob (see Section 3.6.2)] and sample preparation for subsequent analyses was performed every second day of the experiment [days: 0, 2, 4, 6, and 8; ( $n=48$ cobs/day)]. At each day of experimental procedures, 16 cobs [4 (naked cobs stored at 2°C) + 4 (naked cobs stored at 7°C) + 4 (window stripped cobs stored at 2°C) + 4 (window stripped cobs stored at 7°C)] were processed as raw and the remaining 32 cooked in a Panasonic, NN-CD757W microwave for 5 and 10 min with the same number of replicates as described for fresh cobs. The protocol used to cook the sweetcorn cobs and the procedures performed after cooking and until snap-freezing with liquid nitrogen were as described in Section 7.3. In addition, in the current experiment the water which remained in the microwavable bowl after cooking, was collected and assessed for TSS (Section 3.6). Cobs were then stored at -80°C instead of -40°C used for storage of samples targeting in other analytes, so that any possibility of carotenoid and L-ascorbic acid degradation would be reduced. The procedure followed is also presented in a flow chart (Figure 8.1). Methodology of the experiment is described in Chapter 3 and includes: measurement of CO<sub>2</sub> concentration in sweetcorn packs, calculation of moisture content and weight loss, measurements of colour parameters in kernels and husks, total soluble solids, maximum compressive load and analyses for sugars, total phenolics, ferulic acid, L-ascorbic acid, carotenoids and total antioxidant activity.

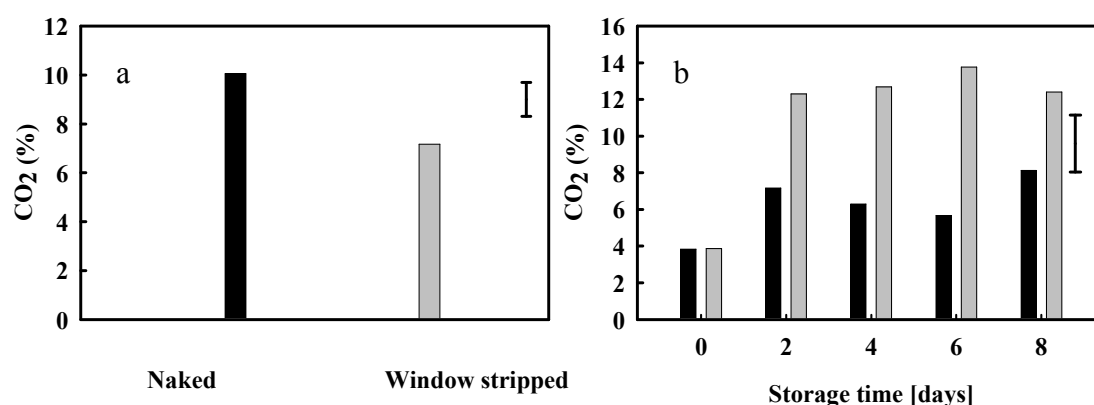


**Figure 8.1:** Flow chart of the Materials and Methods Section (8.2)

## 8.3 Results and discussion

### 8.3.1 CO<sub>2</sub> concentration and weight loss

The existence of husks on cobs during storage and the interaction of storage time and temperature had significant effects on weight loss and CO<sub>2</sub> concentration in packs containing sweetcorn cobs of the cv. 6800 (Figure 8.2 and 8.3). The relevant statistical analyses are shown in Appendix B and the level of significance was 5%. Unsurprisingly, results obtained from the CO<sub>2</sub> and weight loss measurements indicated that sweetcorn cobs stored at 2°C, had significantly lower weight loss and lower CO<sub>2</sub> concentration in packages in comparison with cobs stored at 7°C (Figure 8.1, 8.2 and 8.3), in agreement with the statement that generally the optimum storage temperature is close to 0°C (Kader, 2004; Riad, 2004). Besides, it is well-known that metabolic rates and biological reactions increase with increasing temperature. Furthermore, results indicated that CO<sub>2</sub> (%) in packages with naked cobs was greater than in packages contained window stripped cobs and accordingly naked cobs lost mass at a higher rate.

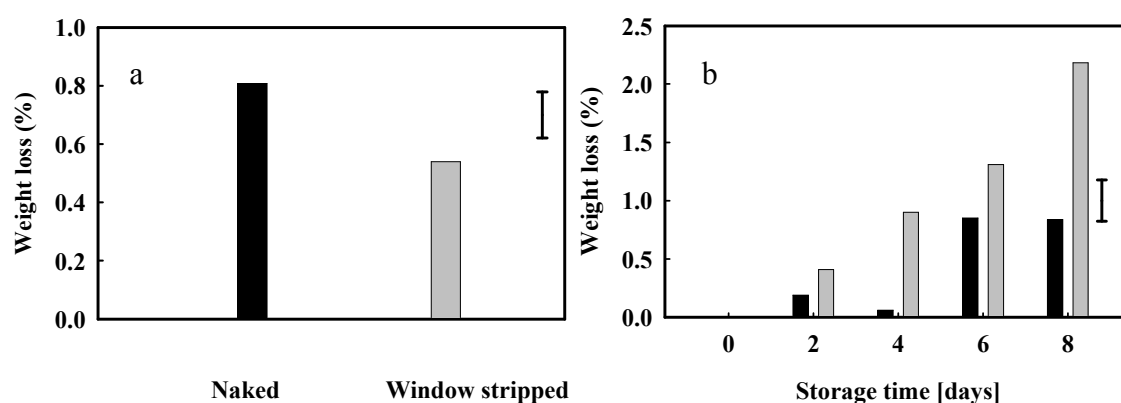


**Figure 8.2:** (a) Mean CO<sub>2</sub> (%) concentration in packs of naked vs. window stripped cobs and (b) of cobs stored at (■) 2°C and (■) 7°C during 9 days of storage. The bar indicates the l.s.d. of (a) format and (b) of combined storage temperature and storage time.

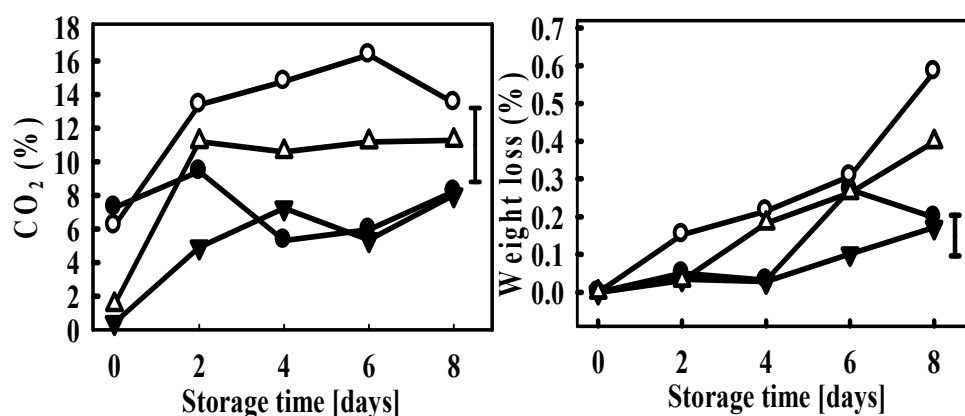
Rate of weight loss was also increased significantly over storage time (Figure 8.3b and 8.4b). These results might be explained by considering that window stripped cobs, are covered by husks and therefore may have promoted a higher relative humidity



around kernels (Shannon, 1968) and in doing so may have created a localised modified atmosphere which may lead to reduced respiration rate of kernels. Results are in agreement with Tewfik and Scott (1954) who also reported that respiratory rates of sweetcorn were lower when their husks had not been removed. The authors also stated that the influence of storage temperature (0, 3.3, 6.7 and 22.2°C) at respiration rate, over a period of 7 days was more important and in particular that respiration rate was increased with increasing temperature.



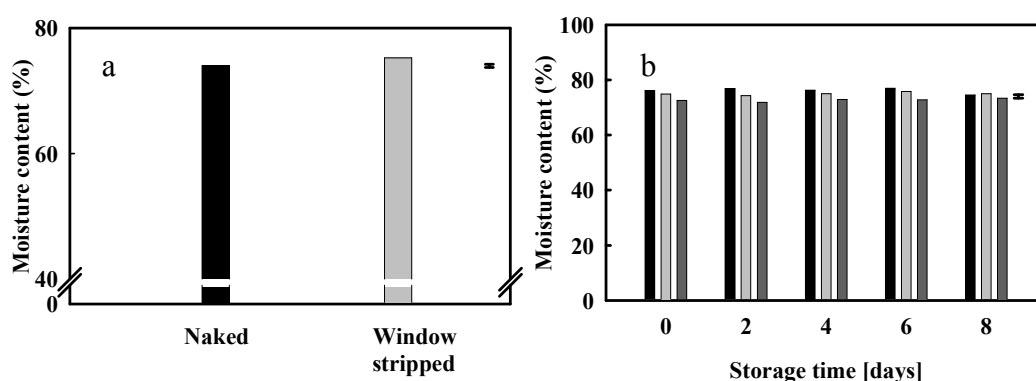
**Figure 8.3:** (a) Weight loss (%) mean values of naked vs. window stripped cobs and (b) of cobs stored at (■) 2°C and (■) 7°C during 8 days of storage. The bar indicates the l.s.d. of (a) format and (b) of combined storage temperature and storage time.



**Figure 8.4:** Mean CO<sub>2</sub> (%) concentration and weight loss (%) mean values of naked cobs stored at (●) 2°C and (○) 7°C and window stripped cobs stored at (▼) 2°C and (Δ) 7°C respectively. The bars indicate the l.s.d. of combined storage temperature, storage time and format of the cobs.

### 8.3.2 Moisture content of kernels

Moisture content is a very important factor not only in preharvest life of sweetcorn but also during postharvest storage period, affecting the appearance and textural characteristics of kernels and the chemical profile of corn (Szymanek *et al.*, 2003). In the present work, the existence or absence of husks and the interaction of cooking time and storage period had significant effects on moisture content of sweetcorn cv. 6800 cobs while storage temperature did not. In particular, moisture content decreased with increased cooking time. According to the results, as shown in Figure 8.5b, fresh cobs had higher moisture content than cobs cooked for 10 min and occasionally higher than cobs cooked for 5 min. Furthermore, cobs stored without husks had lower moisture content than window stripped cobs (Figure 8.5a). In Figure 8.5b, it is shown that no significant changes in moisture content of the cobs examined occurred over time of storage, which indicates that quality was retained over the 8 days of storage.



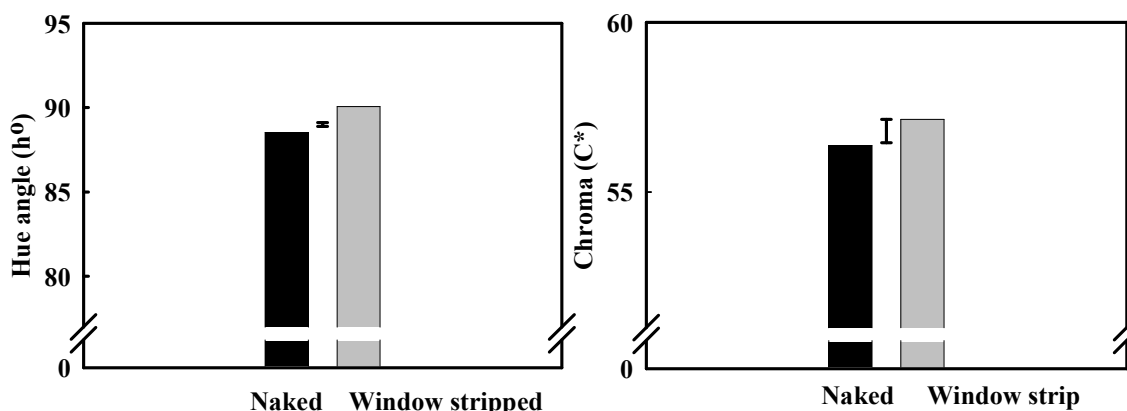
**Figure 8.5 (a):** Mean moisture content (%) values of naked vs. window stripped cobs and **(b)** of (■) raw cobs and cobs cooked for 5 min (■) and 10 min (■) during 8 days of storage. The bars indicate the l.s.d. of (a) format and (b) of combined storage time and cooking time.

### 8.3.3 Colour characteristics

#### 8.3.3.1 Colour characteristics of kernels

Cooking time had significant effects on hue angle of the sweetcorn cobs examined. Generally fresh cobs had lower hue angle values and therefore were less

yellow than cooked cobs, while no significant differences were observed between different cooking times and between cobs stored under different temperature regimes. Furthermore, hue angle and chroma values were higher for window stripped cobs in comparison to naked cobs (Figure 8.6). Figure 8.7 displays the colour of kernels of fresh naked and window stripped cobs.



**Figure 8.6:** Mean hue angle and chroma values of naked vs. window stripped cobs. The bars indicate the l.s.d. of format.

Cooked cobs had higher hue angle values (Figure 8.9) turning colour to yellow-orange (Figure 8.7) in agreement with Barrett *et al.* (2000). Results were also in agreement with Perkins-Veazie *et al.* (1994) who suggested that blanched samples were darker than un-blanched samples. Colour changes in kernels might be related to the activity of the enzyme peroxidase (POD). In more detail, when POD is inactivated, the potential damage of carotenoids is reduced (Baloch *et al.*, 1977) and therefore colour was affected according to the carotenoid profile of samples. In addition, it has been found that in water blanched corn kernels, POD isoforms were better retained than after microwave blanching (Boyes *et al.*, 1997). Therefore, the lack of changes in colour could also be explained by a combination of efficient inactivation of peroxidase due to microwave cooking and of the relationship between carotenoid content and colour (see Section 8.3.8) as a strong correlation between hue angle and carotenoids was found (Table 8.1).

Mean values of lightness obtained from the interaction of format, storage temperature, cooking and storage time, did not indicate significant differences between cobs cooked for 5 and 10 min and neither between different formats (naked vs. window

stripped) and storage temperature. However, lightness values were higher for fresh cobs than from cooked ones (Figure 8.9b and 8.9e). Hence, the visual quality of the cobs tested in terms of colour lightness was not affected by cooking time and format of the cobs during storage.

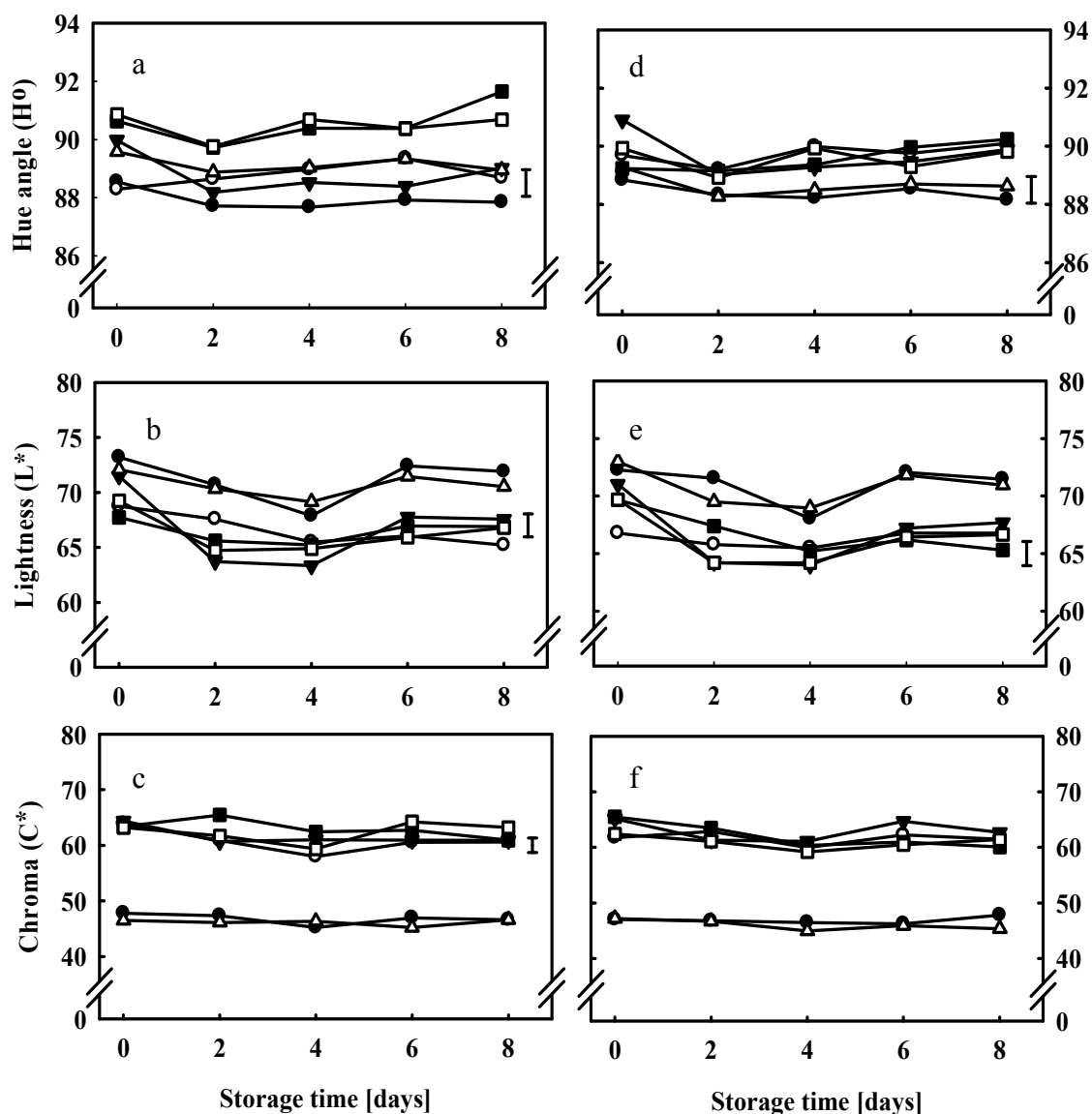
On the other hand, mean values of chroma were significantly lower for fresh cobs when compared to cooked cobs; while when format was the source of variation, window stripped cobs had higher chroma values than naked cobs. Hence fresh cobs had lower colour intensity. Storage temperature was rarely a source of variation but when it occurred, showed that at 2°C chroma values were higher compared to cobs stored at 7°C (Figure 8.9c and 8.9f). Chroma values can probably be explained by the in reverse relationship with moisture content and the negative correlation with total carotenoids (correlation coefficient: -0.358, Table 8.1) as a result of dilution (Xu *et al.*, 2010).



**Figure 8.7:** Raw and cooked (for 5 and 10 min) sweetcorn cobs of cv. 6800.



**Figure 8.8:** Naked and window stripped sweetcorn cobs of cv. 6800.



**Figure 8.9 (a):** Mean hue angle ( $h^\circ$ ), **(b)** Lightness ( $L^*$ ) and **(c)** chroma ( $C^*$ ) values of raw (●) and cooked sweetcorn for (○) 5 and (▼) 10 min stored without husks and raw (Δ) and cooked sweetcorn (■) 5 and (□) 10 min stored with husks for 8 days. **(d)** Mean hue angle ( $h^\circ$ ), **(e)** Lightness ( $L^*$ ) and **(f)** chroma ( $C^*$ ) values of raw (●) and cooked sweetcorn for (○) 5 and (▼) 10 min stored at 2°C and raw (Δ) and cooked sweetcorn (■) 5 and (□) 10 min stored at 7°C for 8 days. The bars indicate the l.s.d. of combined storage time, cooking time and format or temperature respectively.

**Table 8.1:** Correlation coefficients between quality parameters

		Correlation coefficients							
H <sup>1</sup>	1.000***								
L <sup>2</sup>	0.018	1.000***							
C <sup>3</sup>	0.239***	-0.382***	1.000***						
T.P. <sup>4</sup>	-0.075	0.350***	-0.089	1.000***					
T.A.A. <sup>5</sup>	-0.077	-0.03	0.024	0.098	1.000***				
T.C. <sup>6</sup>	-0.456***	0.226***	-0.358***	0.149*	0.166*	1.000***			
Lutein	-0.416***	0.250***	-0.297***	0.176**	0.173**	0.956***	1.000***		
Zeaxanthin	-0.288***	0.016	-0.312***	-0.025	0.043	0.502***	0.226***	1.000***	
L-A.A. <sup>7</sup>	0.028	0.276***	-0.075	0.007	0.188**	0.108	0.126	-0.011	1.000***
T.S. <sup>8</sup>	-0.027	0.063	-0.049	-0.093	-0.065	-0.019	0.019	-0.120	0.178**
Ferulic acid	-0.178**	-0.223***	-0.017	-0.064	0.017	0.107	0.077	0.127*	-0.107
									-0.200**
									1.000***
H <sup>1</sup>	L <sup>2</sup>	C <sup>3</sup>	T.P. <sup>4</sup>	T.A.A. <sup>5</sup>	T.C. <sup>6</sup>	Lutein	Zeaxanthin	L-A.A. <sup>7</sup>	T.S. <sup>8</sup> Ferulic acid

<sup>1</sup> Hue angle, <sup>2</sup> Lightness, <sup>3</sup> Chroma, <sup>4</sup> Total phenolics, <sup>5</sup> Total antioxidant activity, <sup>6</sup> Total carotenoids, <sup>7</sup> L-ascorbic acid, <sup>8</sup> Total sugars

\* Strong correlation at significant level 0.05, \*\* Strong correlation at significant level of 0.01, \*\*\* Strong correlation at significant level of 0.001. The level of correlation was considered significant when the calculated correlation coefficients were lower than -0.1266/-0.1667 and -0.2136 or greater than 0.1266/0.1667 and 0.2136 for a significance level of 0.05/0.01 or 0.001 respectively.

### 8.3.3.2 Colour characteristics of husks

Means of hue angle obtained from measurements in the husks were higher for cobs stored at 2°C when compared to 7°C and in both cases decreased over time (less green colour) indicating postharvest deterioration (Riad and Brecht, 2003). The pattern that lightness followed was arbitrary, while mean values of chroma were occasionally lower in cobs stored at 7°C and decreased over time (Table 8.2).

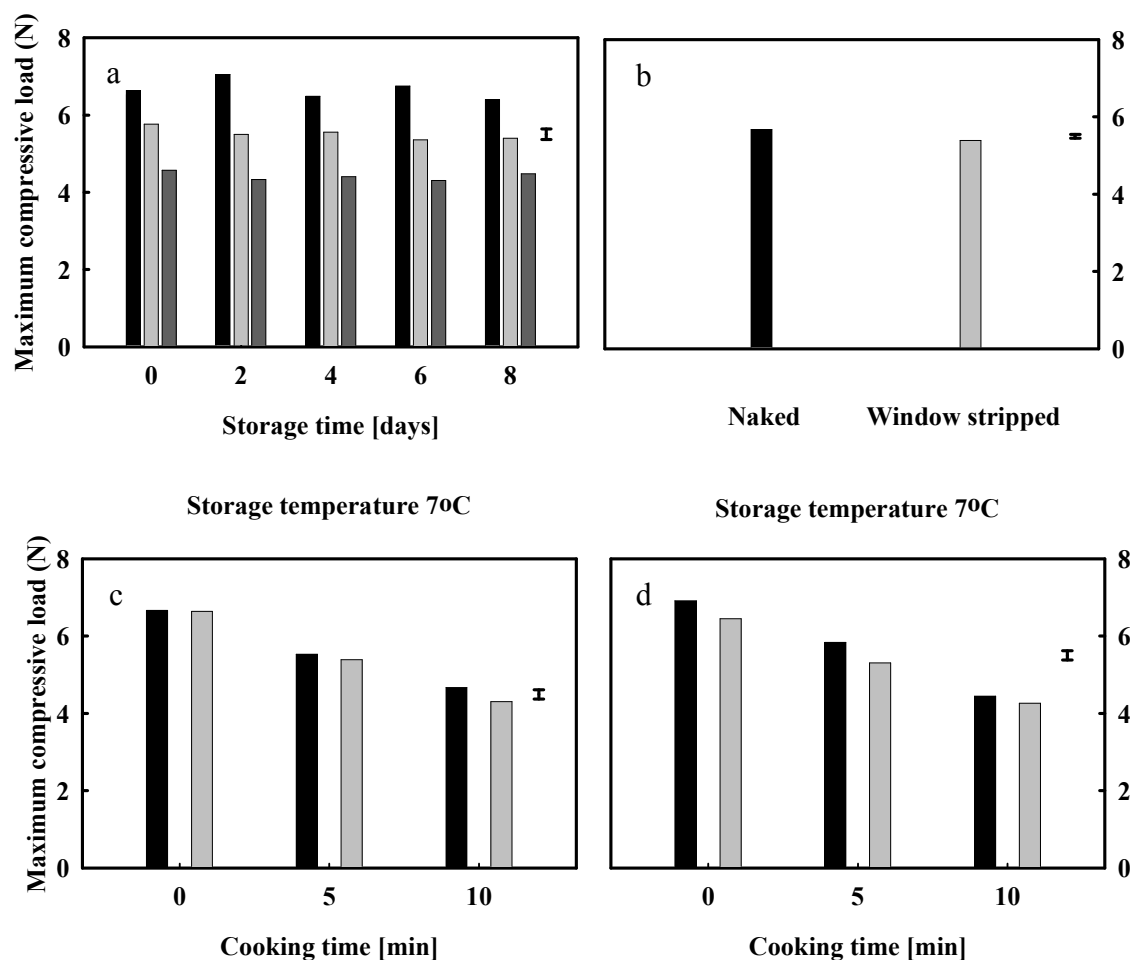
**Table 8.2:** Mean hue angle (h°), lightness (L\*) and chroma (C\*) values derived from sweetcorn husks of cv. 6800 stored at 2 and 7°C for 8 days. L.s.d. indicates the least significant difference of combined storage temperature and storage time.

Colour of husks						
	2°C	7°C	2°C	7°C	2°C	7°C
Day	Hue angle (h°)		Lightness (L*)		Chroma (C*)	
0	114.26a	113.18b	59.13bc	59.99bc	40.64a	38.66abc
2	111.88abcd	108.49de	68.7a	62.83abc	39.37ab	36.47bc
4	112.79abc	109.39cde	62.65abc	67.11a	37.8abc	35.83c
6	112.8abc	108.13e	59.97bc	60.27abc	38abc	35.99bc
8	110.62abcde	108.94de	66.55ab	65.49abc	36.8bc	36.75bc
L.s.d ( $P<0.05$ )	3.702		8.095		3.531	

### 8.3.4 Firmness

Texture is a major quality attribute of sweetcorn closely related to moisture content of kernels, water soluble polysaccharides content and pericarp tenderness (Wann *et al.*, 1971; Szymanek *et al.*, 2003). According to Azanza *et al.* (1996a) the lower the force required to crush kernels, the more tender and desirable they are to consumers. Most commonly, sensory panels evaluate kernel tenderness in opposition to

toughness and kernel firmness in opposition to kernel mushiness (Perkins-Veazie *et al.*, 1994).



**Figure 8.10:** (a) Maximum compressive load of raw sweetcorn cobs (■) and cooked cobs for 5 min (■) and 10 min (■) stored over a period of 8 days, (b) of cobs stored under different format conditions (naked vs. window stripped) and of raw and cooked cobs (for 5 and 10 min) stored as naked (■) and window stripped (■) under different storage temperatures [(c) 2°C and (d) at 7°C]. The bars indicate the l.s.d. of (a) combined cooking time and storage time, (b) of format and (c and d) of combined format, storage temperature and cooking time.

Results derived from the measurements of maximum compressive load indicated that raw sweetcorn cobs were significantly firmer than cooked cobs and cobs cooked for 5 min were firmer than those cooked for 10 min (Figure 8.10a, c and d). It is believed that blanching improves sweetcorn texture (Collins *et al.*, 1996). However in order to



suggest a cooking time leading to texture closer to consumer preferences, the contribution of sensory panels for such conclusion would be essential. Furthermore the evaluation of the interaction of storage time, storage temperature and format of the cobs indicated that occasionally cobs stored at 2°C were firmer than cobs stored at 7°C (Figure 8.10c and d) and that naked cobs were firmer than window stripped cobs (Figure 8.10b). It was expected that a decline in firmness would be observed with increasing temperature, due to the negative correlation between these two parameters (Bourne, 1982) as explained in Section 2.5.2. On the other hand, transpiration of water from kernels is higher than transpiration of water from husks (Showalter, 1963) which in turn means that naked cobs have lower moisture content (Figure 8.5a) and hence greater firmness compared to window stripped cobs.

### 8.3.5 Sugars and total soluble solids

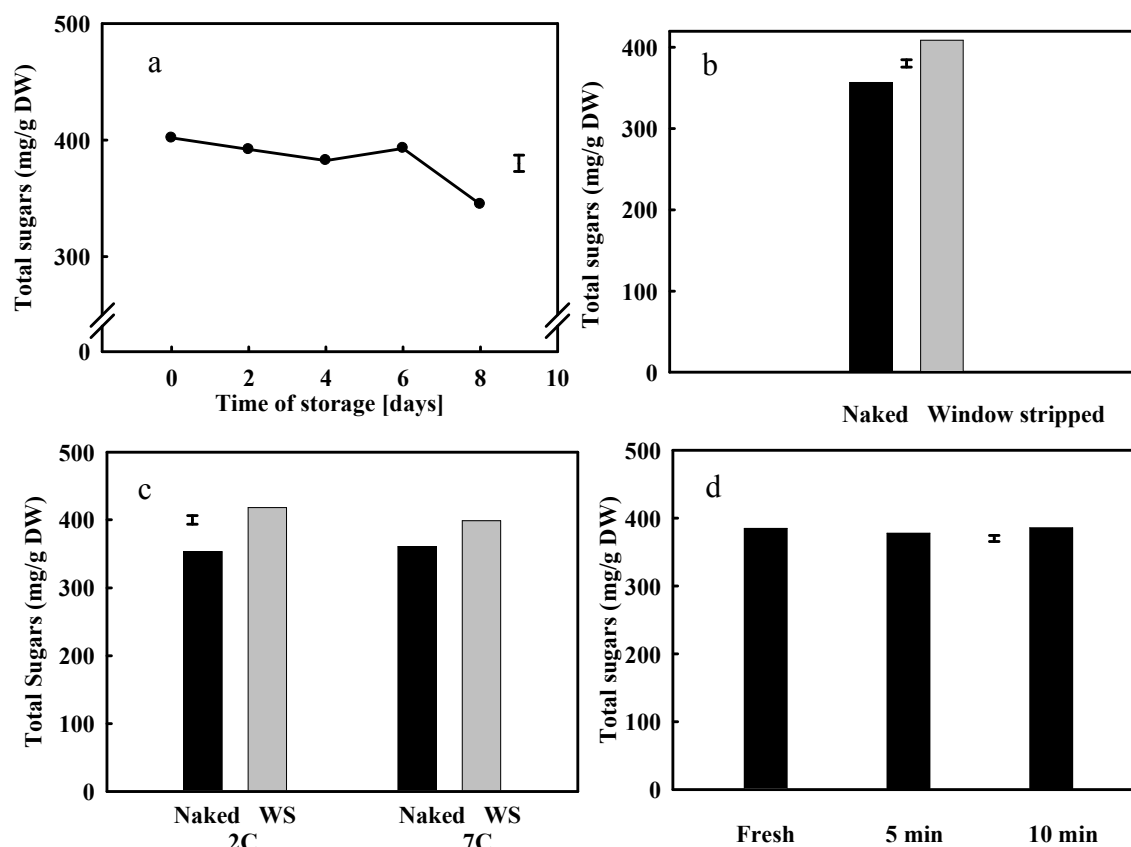
Format of the cobs (naked, window stripped), storage time, and the interaction of format and temperature had significant effects in total sugar content while cooking time did not (Figure 8.11). Sucrose content was not affected significantly by cooking time (Table 8.3). On the other hand, glucose and fructose content of raw cobs were higher than for cooked cobs. However, no significant differences were observed between cobs cooked for 5 and 10 min (Table 8.3). Barrett *et al.* (2000) did not find any effect of blanching time [(0, 2, 4, 6, and 8 min) in blanch steamer] in concentration of individual sugars. Sucrose dominated among sugars found with a correlation coefficient to total sugars 0.989 ( $P < 0.05$ ). These effects indicated that window stripped cobs had significantly higher total sugar content. Riad *et al.* (2003) found that total soluble solids content of fresh-cut sweetcorn kernels was reduced during storage period and sugar loss after cooking. However fresh-cut kernels are more perishable to intact kernels and the possibility of sugar loss due to dilution during cooking. In agreement with the results of the current work, it has been reported that sugar loss in sweetcorn kernels after blanching for up to 10 min was not significant (Szymanek *et al.*, 2003). However, it cannot be said that sugar content was predictably affected by the storage temperature, but when occurred high sugar content was favoured by storage at 2°C (Figure 8.11). This indication was expected as 2°C is closer to the optimum storage temperature (0°C)

of sweetcorn (Riad, 2004), which was not used as according to the objectives since the purpose of this experiment was to simulate commercially used handling conditions. Hence, in terms of sugars, storage temperature of 2°C is more beneficial than 7°C as contributes better to the retention of sugar content. However the differences between 2 and 7°C were not as great as expected as for both temperatures sugar content was well maintained. Sweetcorn cobs of cv. 6800 stored with their husks also maintained sugar content better when compared to cobs that their husks have been removed.

Courter *et al.* (1988) suggested that browning of sweetcorn kernels after cooking might be a result of Maillard reaction (reaction between free amino acids and sugars), while in a later study (Riad *et al.*, 2003) this hypothesis was rejected. However, no evidence of such incidence was obtained from the current results herein as no significant correlation between sugars and colour parameters was found. In addition, sugar content of the cobs examined did not appear to be strongly correlated with L-ascorbic acid and therefore the hypothesis of regulation of vitamin C content by sugars-precursors of its biosynthesis was not supported (Massot *et al.*, 2010).

**Table 8.3:** Sucrose, glucose and fructose content of sweetcorn cobs [(mg/g DW) over the 8 days of storage] of cv. 6800 as affected by the interaction of storage temperature, format of the sweetcorn cobs and cooking time. L.s.d. refers to the least significant difference of combined storage temperature, format and cooking time.

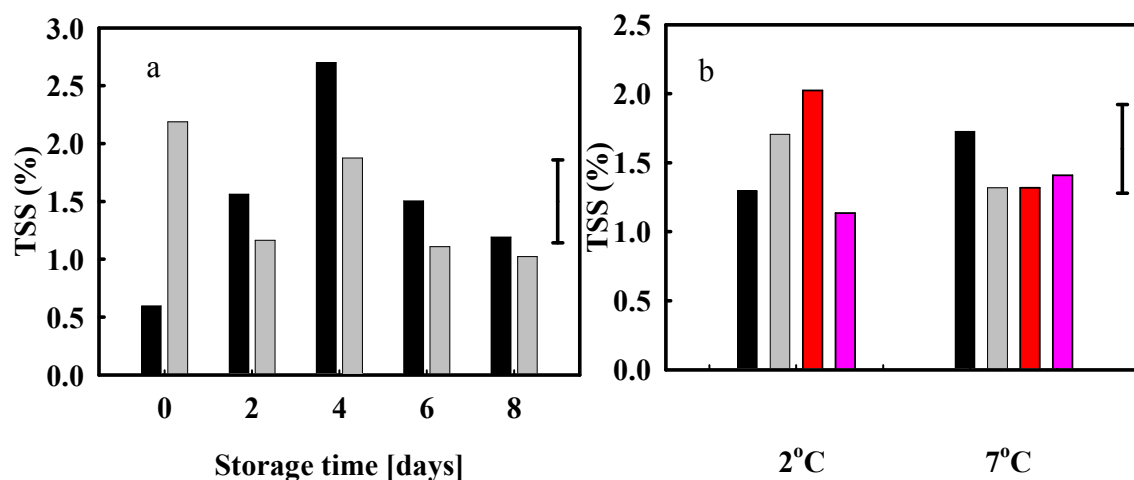
		Sucrose (mg/g DW)				
Temperature (°C)		2		7		
Cooking time (min)	0	5	10	0	5	10
Naked	297.3d	305.4d	312.1d	304.5d	313.7d	314.1d
Window stripped	355.9ab	348.5ab	365.4a	347.7ab	336.8c	342.7b
L.s.d.: 19.00						
		Glucose (mg/g DW)				
Temperature (°C)		2		7		
Cooking time (min)	0	5	10	0	5	10
Naked	34.31cd	29.58de	31.33d	36.02c	30.42de	31.63de
Window stripped	43.97a	39.97b	40.4ab	40.62ab	37.45bc	37.05bc
L.s.d.: 3.604						
		Fructose (mg/g DW)				
Temperature (°C)		2		7		
Cooking time (min)	0	5	10	0	5	10
Naked	18.04bcd	15.63ef	15.69e	19.26b	15.58f	16.52def
Window stripped	22.25a	19.98b	18.53bcd	19.51bc	17.77cde	17.78cde
L.s.d.: 2.107						



**Figure 8.11:** Mean total sugar content (mg/g DW) of (a) all cobs during storage, (b) of naked vs. window stripped, (c) naked vs. window stripped cobs stored at 2°C and at 7°C and (d) of fresh cobs and cobs cooked for 5 min and 10 min. The bars indicate the l.s.d. of storage time, format, combined format and storage temperature and cooking time, respectively.

Total soluble solids (TSS) content was not correlated to any of the individual sugars analysed ( $P < 0.05$ ). Occasionally, TSS in the water which remained in the microwavable bowl was higher when cobs had been cooked for 10 min rather than 5 min which probably indicate greater TSS leaching with increased cooking time (Figure 8.12a). Results derived from the interaction of storage temperature, cooking time and format (naked vs. window stripped) also had significant effects in TSS content. In particular, TSS measured in water collected from naked cobs cooked from 10 min was significantly higher compared to naked cobs cooked for 5 min. In addition, cobs stored at 2°C released greater TSS when cooked for 5 min when compared to cobs stored at

7°C (Figure 8.12b). It is worth noting that TSS content was not high (Figure 8.12) and that it is not always considered as an appropriate method for estimation of sugars in several crops (Gine Bordonaba, 2010).



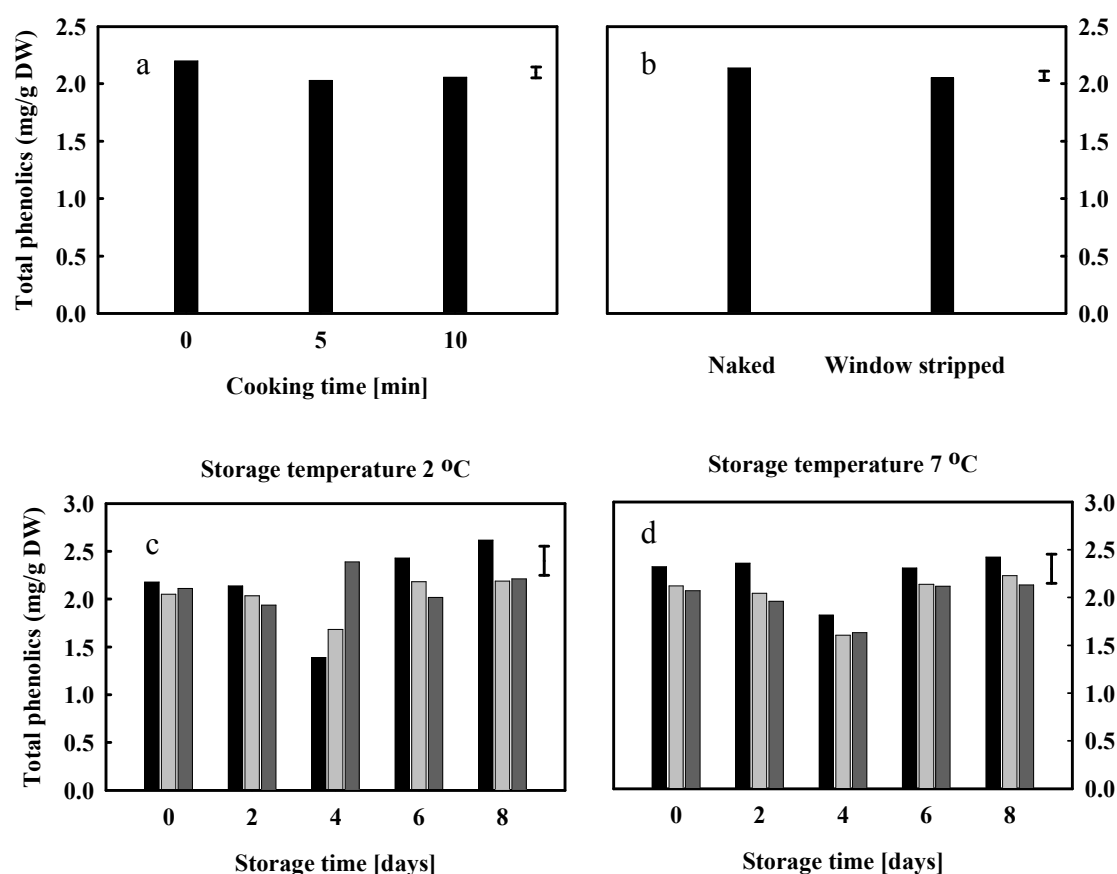
**Figure 8.12:** Mean total soluble solid (TSS) content (%) of (a) cooked sweetcorn for (■) 5 and (■) 10 min and (b) of cooked (5 min) sweetcorn stored at 2°C as naked (■) and window stripped (■) and of cooked (5 min) sweetcorn stored at 7°C as naked (■) and window stripped (■). The bars indicate the l.s.d. of combined (a) cooking time and storage time and (b) of combined format, storage temperature and cooking time.

### 8.3.6 Phenolics and Total Antioxidant Activity

#### 8.3.6.1 Accuracy of the Folin-Ciocalteu assay for total phenolics evaluation

Total phenolic content declined under cooking procedures, but no significant differences were observed in the total phenolic content between cobs cooked for 5 and 10 min (Figure 8.13a). These results are in agreement with previous studies where authors reported that upon cooking the total soluble phenolic content of fresh-cut sweetcorn kernels was decreased (Riad *et al.*, 2003). In another study, it has been suggested that upon heat-treatment free total phenolic content was increased and bound phenolic content decreased (Dewanto *et al.*, 2002) which might help to explain the results of the current work, as with the method used [Folin-Ciocalteu, (Section 3.11.1)]

it is speculated that both bound and free phenolic compounds were measured. Furthermore, results from the interaction of format, cooking time and storage temperature indicated that occasionally cobs stored at 7°C had higher total phenolic content than cobs stored at 2°C (Figure 8.13c and Figure 8.13d). In addition, naked cobs had higher total phenolic content than window stripped cobs (Figure 8.13b). Considering the potential antioxidant properties of phenolic compounds due to the anti-radical and other properties they have (Lopez-Martinez *et al.*, 2009), it can be assumed that raw cobs have potentially greater amounts of antioxidant compounds and that kernels of naked cobs and cobs stored at 7°C might reveal higher favourable bioactive properties in comparison to window stripped cobs or cobs stored at 2°C.

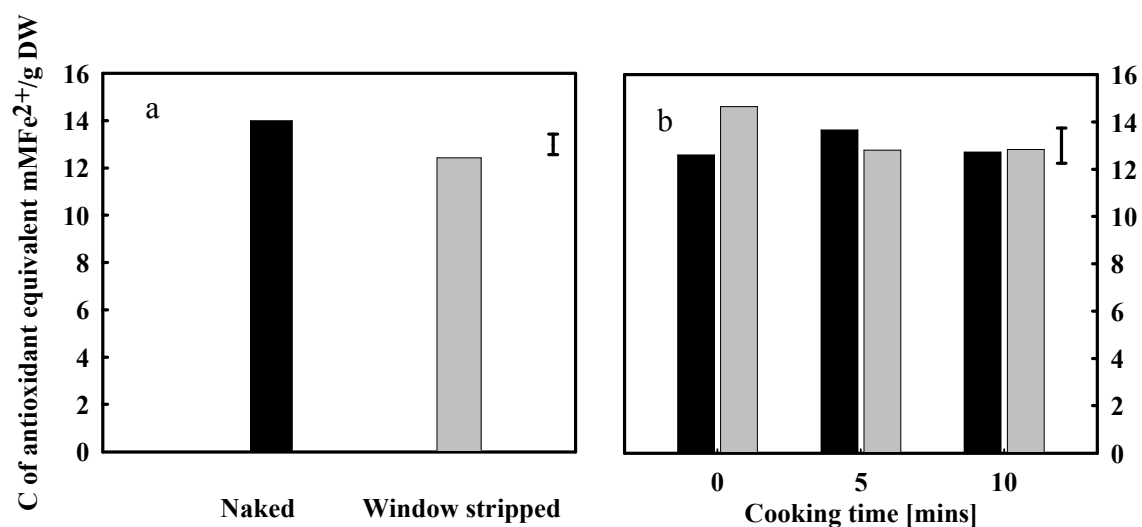


**Figure 8.13:** (a) Total phenolics concentration (mg/g DW) of raw and cooked cobs for 5 min and 10 min, (b) of cobs stored under different format conditions (naked vs. window stripped) and of raw (■) and cooked cobs for 5 min (▒) and 10 min (■) stored at 2°C (c) and at 7°C (d). The bars indicate the l.s.d. of (a) cooking time, (b) format and (c and d) of combined storage temperature and cooking time.

Results obtained and absence of correlation with ferulic content and colour indicate that the method was inappropriate for the measurement of total phenolics in the sweetcorn cobs examined. The inaccuracy of the method in the current study probably relies on the very high concentrations of sugars and the presence of ascorbic acid which both interfere in the measurement of total phenolics (Atkinson *et al.*, 2005). Besides Folin-Ciocalteu assay measures the sum of polyphenols in the samples but does not quantify individual phenolic compounds of special interest in terms of antioxidant activity or compounds that turn to a more brownish colour after auto-oxidation (Riad, 2003). From the results herein, it is therefore advised that the Folin-Ciocalteu assay is not used for sweetcorn.

#### **8.3.6.2 Suitability of ferric ion reducing antioxidant power assay for the evaluation of total antioxidant activity**

Knowledge of the antioxidant capacity is fundamental since it reveals the potential health benefits. In the current study, total antioxidant activity of the sweetcorn cobs tested was affected by the format of the cobs and by the interaction of cooking time and storage temperature (see Appendix B). Changes in total antioxidant activity were not correlated with total phenolic content (Table 8.1) probably due to the poor accuracy of the methods used for the measurements of these compounds (Chapter 3). However, generally, results were inconsistent and did not clarify which of the postharvest factors of storage examined were beneficial in terms of total antioxidant activity.



**Figure 8.14:** (a) Antioxidant activity as expressed to  $\mu\text{MFe}^{2+}/\text{g DW}$  of naked raw and window stripped cobs and (b) of raw and cooked cobs for 5 min and 10 min stored at 2°C (■) and at 7°C (■). The bars indicate the l.s.d. of (a) format and (b) of combined storage temperature and cooking time.

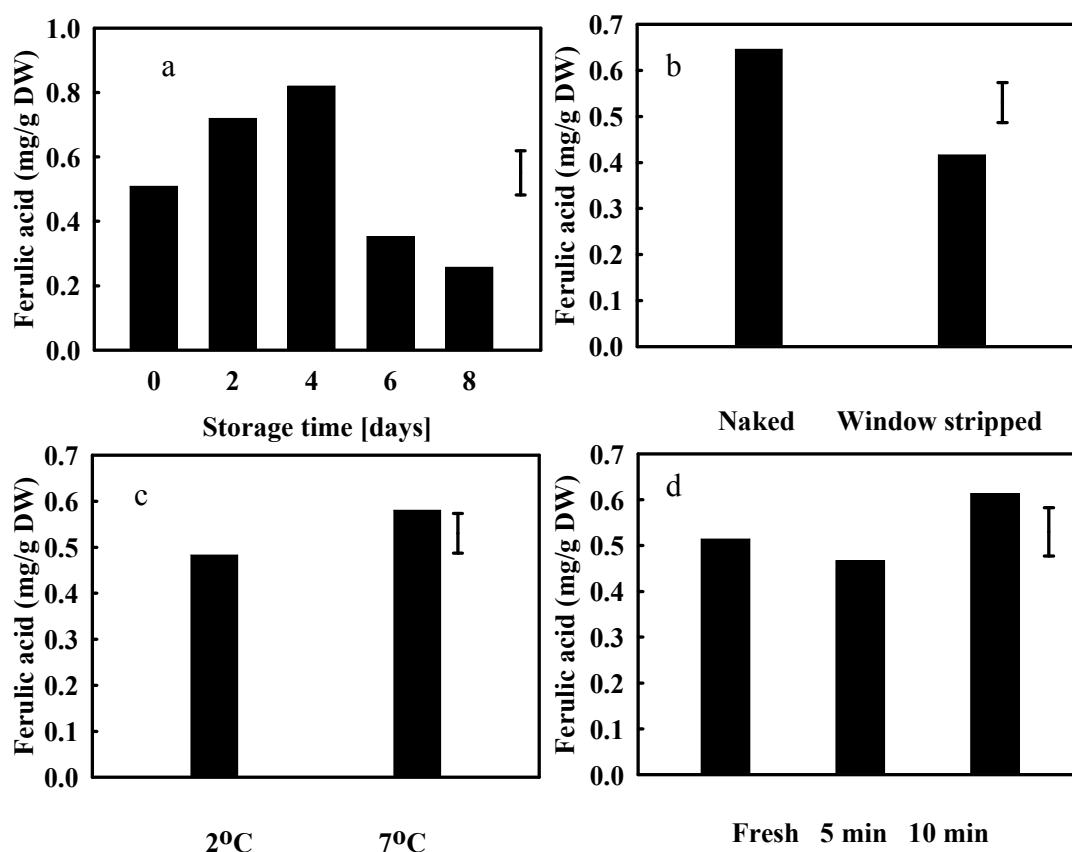
### 8.3.6.3 Can colour changes be useful for a rapid evaluation of changes in ferulic acid content?

Ferulic acid content was reduced significantly after day 4 of the experiment while naked cobs had greater ferulic acid content than window stripped cobs (Figure 8.15a and Figure 8.15b). Results indicated that cobs cooked for 10 min had the highest ferulic acid content (Figure 8.15d). In addition, cobs stored at 2°C had lower ferulic acid content than cobs stored at 7°C (Figure 8.15c). In previous studies, it has been reported that free ferulic acid concentration increased and bound ferulic acid content decreased upon thermal processing and heating time. Increased amounts of free ferulic acid might be explained by cell-wall ester glycosides that release ferulic acid under thermal processing and in that case cooking (Dewanto *et al.*, 2002).

The increased ferulic acid content was not correlated with total phenolic content; contributing to the inexplicit accuracy of the Folic-Ciocalteu assay for the measurement of total phenolics. In contrast, reduction of ferulic acid and therefore its antioxidant



activity can probably be explained by conjugation of ferulic acid with fatty acids and mainly sugars that are found in very high quantities in sweetcorn (White and Xing, 1997). Furthermore, ferulic acid content was correlated with hue angle and lightness (Table 8.1), and therefore colour changes can be used for a rapid indicator of the changes occurring in ferulic acid and total phenolic content.



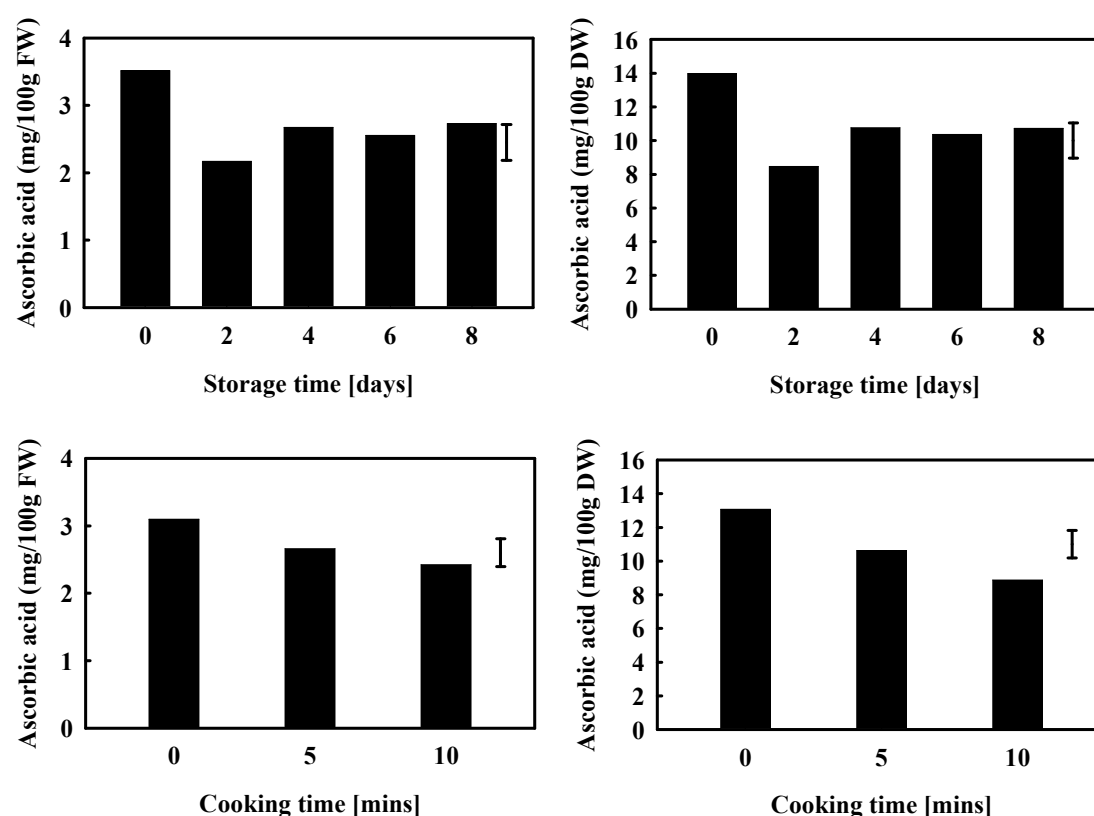
**Figure 8.15:** (a) Ferulic acid concentration (mg/g DW) during a storage period of 8 days, of (b) naked and window stripped cobs, (c) of cobs stored at 2°C vs. cobs stored at 7°C and (d) of fresh and cooked cobs for 5 min and 10 min. The bars indicate the l.s.d. of storage time, format, storage temperature and cooking time respectively.

### 8.3.7 L-ascorbic acid

L-ascorbic acid content of the cobs examined, declined over storage time on both dry and fresh weight basis (Figure 8.16). Furthermore, L-ascorbic acid content of

raw cobs was significantly greater than that of cooked cobs (Figure 8.16). On the other hand, L-ascorbic content of cobs stored at different temperature and of cobs stored under different format condition (naked vs. window stripped) did not differ significantly (see Appendix B).

Any decrease of the L-ascorbic acid might be explained by its conversion to 2, 3-diketoglutonic and dehydroascorbic acid (DHAA). Considering that DHAA is the oxidised form of ascorbic acid and that oxidation occurs rapidly when exposed to heat (Gregory, 1996), a lower concentration of L-ascorbic acid measured in cooked sweetcorn cobs, when compared to raw cobs, was expected. Dewanto *et al.* (2002) also reported loss in ascorbic acid after thermal processing for 10, 20 and 30 min and the relevant decline upon increase of heating time which is not inconsistent with the results of the current study as different cooking times were applied to the sweetcorn samples.



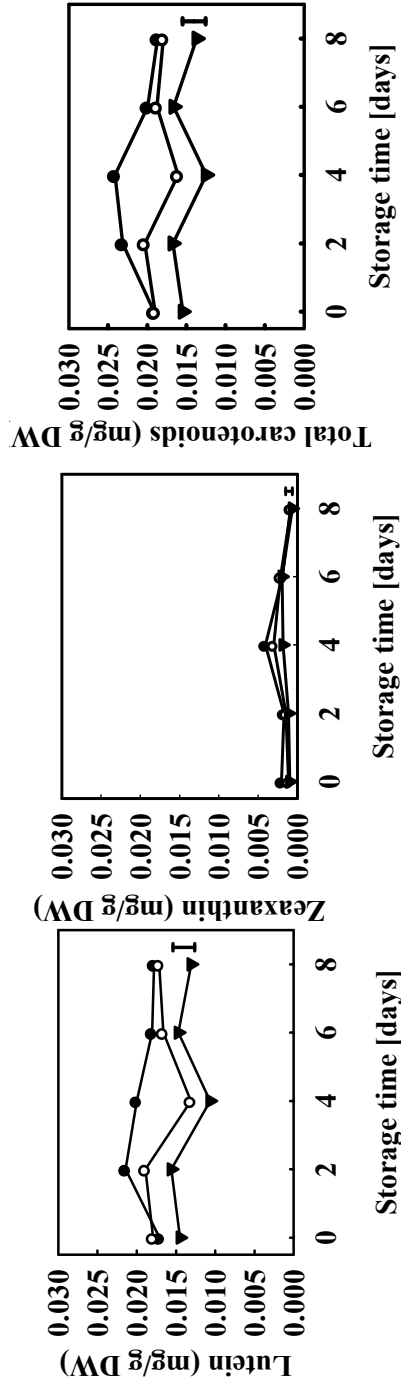
**Figure 8.16:** Mean L-ascorbic acid concentration (mg/100g on FW and DW basis) of sweetcorn cobs during 8 days of storage and mean L-ascorbic acid concentration (mg/100g on FW and DW basis) of raw sweetcorn cobs and cobs cooked for 5 and 10min. The bars indicate the l.s.d. of storage time and cooking time respectively.

### 8.3.8 Effects of storage conditions in carotenoid content

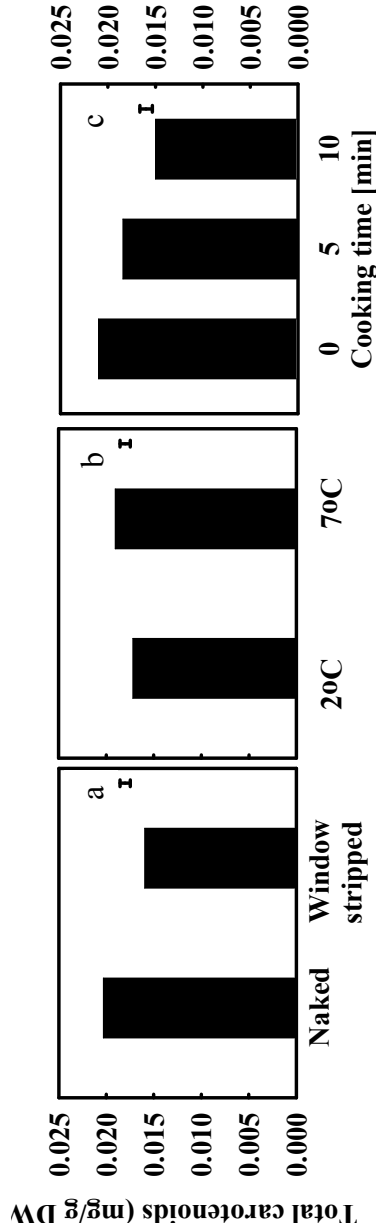
Carotenoids in sweetcorn are found in kernel endosperm with the xanthophylls lutein and zeaxanthin dominating among them. Their antioxidant properties and contribution in prevention of several diseases such as eye degeneration disease render them of great importance for human health (Ribaya-Mercado *et al.*, 2004; Kopsell and Kopsell, 2006). Figure 8.17 and Figure 8.18 display carotenoid content of the cobs examined over a storage period of 9 days on dry weight basis, as generally the changes in moisture content that might occur upon cooking and during storage can be misleading. Besides, for carotenoids and all other compounds examined with single exception that of ascorbic acid, the samples used to examine them were dried and therefore free of moisture.

Naked cobs and cobs of cv. 6800 stored at 7°C had higher carotenoid content than window stripped cobs and cobs stored at 2°C (Figure 8.18). Lutein content was 90-95% of the final carotenoid content as sum of lutein and zeaxanthin (Figure 8.17).

Lutein is the most stable carotenoid against heat (Updike *et al.*, 2003) and this might explain the lack of significant differences that inconsistently occurred between heat treatments. However, it can be said that cooking procedures caused significant decline in carotenoid content in agreement with results previously published by Hart and Scott (1995) who reported a decline of carotenoid content after cooking. Hence, carotenoids and their potential health benefits occur in greater concentrations in uncooked cobs, cobs stored at 2°C and in window stripped cobs when compared to naked. It is worth noting that, the correlations between carotenoids and colour characteristics were stronger than correlations between total phenolics and colour characteristics (Table 8.1) indicating that the visual quality of sweetcorn in terms of colour is potentially indicator of health benefits in terms of carotenoids.



**Figure 8.17.:** Mean lutein, zeaxanthin and total carotenoid (lutein + zeaxanthin) content (mg/g DW) of raw vs. cooked cobs [raw (○), cooked for 5 min (○) and for 10 min (▼)]. The bars indicate the l.s.d. of storage time vs. cooking time.



**Figure 8.18.:** Mean total carotenoid (lutein + zeaxanthin) content (mg/g DW) of (a) naked vs. window stripped cobs, (b) of cobs stored under different temperatures 2°C vs. 7°C and (c) of raw vs. cooked cobs. The bars indicate the l.s.d. of format, storage temperature and cooking time respectively.

## 8.4 Conclusions

This chapter contributed to a better understanding of the physiological and chemical alterations occurring in supersweet sweetcorn upon cooking in the microwave. The null hypothesis that storage temperature, presence of husks, storage time and cooking time does not affect major quality attributes of sweetcorn (sugars and texture) neither its content of basic antioxidant compounds is rejected. It can be concluded that cooking time is an important factor affecting the physiological and biochemical profile of sweetcorn. However, format of the cobs; storage time and storage temperature also resulted in significant changes, mainly in influencing the physiological and antioxidant profile of sweetcorn. On the other hand, storage temperature was more beneficial at 2°C rather than at 7°C for maintaining the initial quality attributes of sweetcorn. Naked cobs had higher amounts of antioxidant compounds in comparison to husk stripped cobs in contrast to their sugar content. Cooking time affected the antioxidant compounds commonly found in corn but without following a specific pattern. Carotenoids and L-ascorbic acid content declined under cooking conditions in contrast to ferulic acid content, contributing to knowledge that may have implications for the ‘five a day campaign’ and consumer perception. Furthermore, the non significant changes in sugar content; indicated that taste preference of consumers would not be affected by cooking. Last but not least, it was shown that colour changes could potentially be used for a rapid evaluation of the changes occurring in ferulic acid and total phenolic content.

## 9. CHAPTER NINE

### Discussion, general conclusions and future recommendations

#### 9.1 Introduction

The importance of sweetcorn relies on its high popularity and market value. Postharvest research mainly targets the extended postharvest quality and shelf life of sweetcorn. Both quality and postharvest life of sweetcorn are greatly dependent on harvest date and the variety (Wong *et al.*, 1994; Kader, 2003; Szymanek, 2009). Maize is a well documented crop. However, there are still deficiencies in the literature, especially regarding target analytes in *shrunk-2* cvs. Furthermore, genotypes commonly consumed and grown in UK have not been studied extensively. Lack of information has also been noted concerning suitability of commonly used methods such as the Folin-Ciocalteu assay for the measurement of total phenolics and the ferric reducing antioxidant power assay as applied to sweetcorn. In addition, effects of cob format during storage, and microwave cooking of supersweet sweetcorn cvs. are not fully elucidated. Storage time and storage temperature have been frequently studied but rarely has the interaction between these factors been previously explored.

In the UK-market, sweetcorn cobs are most commonly sold as naked (de-husked) and window stripped (husks have not been fully removed and have a ‘window-strip’ exposing the kernels). Both formats of the cobs were examined in order to elucidate potential differences in quality parameters between them. The potential effects of length of shank in the quality attributes assessed; were also examined. The interaction between storage period, as a major contributor to perishability and quality deterioration, with other factors of variation such as cooking was also elucidated. The effects of cooking sweetcorn cobs were also assessed according to the duration for which the cobs were microwaved.

Perishability and respiration rate of packaged sweetcorn cobs, rises with increasing temperature, and therefore storage at an appropriate temperature is crucial (Golob *et al.*, 2002; Kader, 2002). The storage temperatures studied in the present work

ranged from 2-16°C. The variety of cobs tested was also considered as a point of variation. The methods used for the analyses conducted, were developed, and/or validated and optimised where possible (see Chapter 3).

The present chapter aims to discuss further the results described in the previous Chapters, to supply recommendations for future work and to highlight the most important conclusions and implications thereof.

## 9.2 Discussion

Especially in earlier years, quality, which is associated with overall consumer acceptability, was mainly assessed through the measurement of sugars and perceived sweetness. Apart from sweetness which is related to taste; texture, moisture content and appearance parameters such as colour of kernels and husks are of high importance (Splitter and Shipe, 1972). Furthermore, in later years, foods containing high levels of antioxidant compounds were promoted as having additional quality parameters. Thus, the quality parameters examined, include measurement of colour, respiration gases, moisture content, firmness, spatial concentrations of individual sugars (sucrose, glucose, fructose), starch and antioxidant compounds, such as L-ascorbic acid, ferulic acid and carotenoids. In terms of the visual quality of sweetcorn, the most important attribute is colour. According to panellists' perception, yellow corns are believed to be preferred by consumers (Splitter and Shipe, 1972). A dark green colour of husks (evaluated only in Chapter 8- Section 8.3.3.2) has been suggested as another criterion for the selection of fresh cobs (Brecht *et al.*, 1990; Szymanek *et al.*, 2003). Furthermore, variables such as moisture content and the respiration gas CO<sub>2</sub> are often useful in understanding other changes which occur during the postharvest life of sweetcorn. In particular, moisture content plays an important role in dilution and might be related to firmness and the concentration of chemical compounds on a fresh weight basis. As an example, moisture loss of *ca.* 2% causes denting in the kernels (Szymanek, 2003) and therefore influences kernels appearance.

### **9.2.1 Appropriateness of total phenolic and ferric reducing antioxidant power assays**

Generally, measurements including firmness and the identification and quantification of starch, carotenoids, ferulic acid and sugars can be characterised as rapid and accurate. However, the appropriateness of the method used for the measurement of total phenolics has been under consideration due to the interference of other substances and especially sugars and ascorbic acid as discussed in Chapter 8. In the present study, the unsuitability of the method was intimated by the absence of strong correlations between total phenolic content and colour changes. The measurement of total phenolics was considered necessary for the estimation of potential contribution of total phenolics to total antioxidant activity and to kernel browning under cooking conditions. A strong correlation between total phenolic content and colour characteristics would provide evidence that browning upon cooking is associated with total phenolic content. A weak correlation between these two parameters would probably indicate that they are poorly related but their relationship is not affected by cooking. Hence, the absolute absence of any correlation between total phenolic content and hue angle in both Experiment 6 and 7 implied the inaccuracy of the Folin-Ciocalteu assay for the measurement of total phenolic content which was an objective of Chapter 3 (Materials and methods).

Similarly, the method used to measure total antioxidant capacity can also be described as inappropriate for sweetcorn. In order to identify the potential degradation of antioxidant compounds, the quantification of the total amount of antioxidants found in corn is required. However, the knowledge of their bioavailability is also necessary. For instance, the bioavailability of carotenoids can be altered by biotic and abiotic factors along with alterations in carotenoid chemistry (Kopsell and Kopsell, 2006). Unfortunately, the results regarding the total antioxidant activity using the ferric ion reducing antioxidant power assay (FRAP) of the cobs examined were inconsistent. The results could not offer a better understanding of supersweet sweetcorn total antioxidant capacity, probably due to the inadequacy of the application of a unique method to measure total antioxidant activity. With the FRAP assay, the ferric ions are reduced by reductants that are not all antioxidants while new free radicals are produced when some antioxidants including ascorbic acid react with ferric ions (Prior and Cao, 1999). This



theory along with the presence of unidentified phenolic compounds (see Chapter 3) which might have the ability to reduce ferric ions, can potentially explain the fluctuation of the total antioxidant activity as measured in Chapter 8.

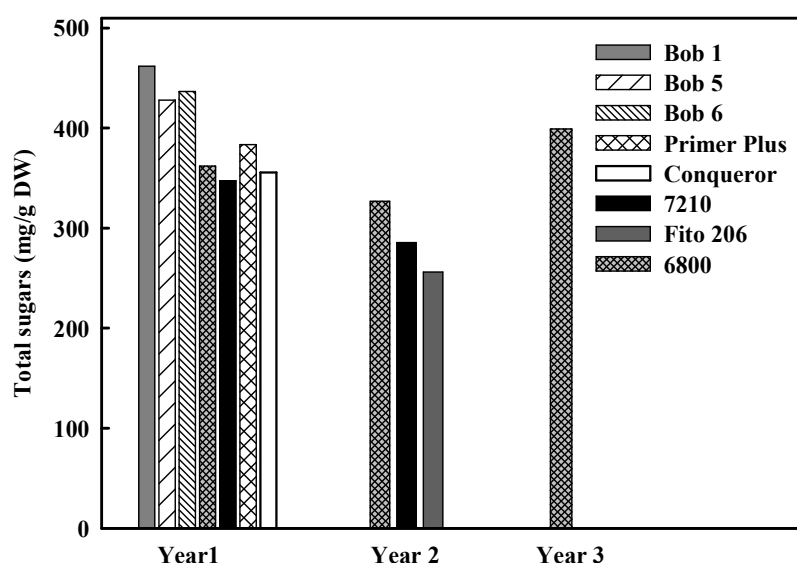
### **9.2.2 Suitability of CA consisting of 8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub> for supersweet sweetcorn cvs. grown in UK**

Visual quality in terms of appearance of husks was maintained in CA storage for 24 days (Chapter 4) as microbial growth in husks can be eliminated by CA conditions (Aharoni *et al.*, 1996) and water loss that is initially observed in husks (Showalter, 1967; Handenburg *et al.*, 1986) can be reduced by CA conditions (Deak *et al.*, 1987). The reduced moisture loss under CA conditions relies on the restricted gas exchange that CA allows (Deak *et al.*, 1987). In Experiment 1 (Chapter 4), CA consisting of 8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub> was tested for its effects on sweetcorn quality. It was observed that moisture content of some cvs. was maintained over time while in some cases moisture content fluctuated as discussed in Chapter 4. Sugar content of the cobs examined was maintained in relatively high levels even when the sugar content was decreasing. Firmness of the cobs was generally maintained during storage, indicating that the CA composition used, achieved one of its major biological effects; to slow down the activity of either enzymes responsible for cell-wall degradation or enzymes that are related to lignification (Kader, 2004). While the CA conditions examined were not compared to air, it can still be said that were appropriate for quality preservation in terms of texture as firmness was maintained during storage. Thus, further research might prove controlled atmosphere storage, consisting of 8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>, to be suitable for long term storage (24 days) of sweetcorn and appropriate as model conditions that should be achieved by appropriate packaging.

### **9.2.3 Influence of genotype on defining postharvest sweetcorn quality**

Genotype, origin and environmental conditions during cultivation are common factors of variation in determining sugar content of the cobs. Quality deterioration of sweetcorn cobs during storage is mainly a result of sugar loss due to the conversion of

sugars to starch (Evensen and Boyer, 1986) and high respiration rate. Analysis of sugars and texture is considered very important for the evaluation of the final consumer acceptability as they are generally found to be strongly associated with the sensory perception of panellists (Splitter and Shipe, 1972; Azanza *et al.*, 1996a). Overall, acceptability of sweetcorn, as assessed by sensory panels, indicates sugars and thus sweetness, as quality characteristics of great importance. Thus, supersweet cultivars are preferred by consumers as their sweetness is higher, in comparison to cultivars which did not include the *sh2* gene (Geeson *et al.*, 1991). Starch content was indeed relatively low according to the results presented, due to the *sh2* gene. Generally, results from all experiments of the current work showed that sucrose dominated, in agreement with the literature referring to *sh2*-corn (Laughnan, 1961; Wong *et al.*, 1994). Sugars of secondary significance were glucose and fructose (Laughnan, 1953; Wong *et al.*, 1994).



**Figure 9.1:** Sugar content of nine of the cvs. studied originating from the UK and Spain during 3 years. Sweetcorn cobs displayed are referred to in Chapter 4 [Year 1: originated from UK], Chapter 6 and 7 [Year 2: originated from UK and Spain respectively] and Chapter 8 [Year 3: originated from Spain]. Values are from fresh cobs only and correspond to the first sampling day of each experiment.

The experiments revealed differences in the sugar content of several cultivars and origins (see Chapter 4, 5 and 6). Part of these results is presented in Figure 9.1, where it

was indicated that the UK-grown Bob cvs. (Chapter 4) had the highest concentrations of sugars compared to all the other genotypes tested. In fact, sugar content of Bob cvs., was almost 2-fold higher than sugar content of cv. Fito 206 (Chapter 6). The significant effects of sweetcorn origin, were also indicated when sugar content of cv. Garrison was found significantly higher when originated from Senegal, rather than the USA (Chapter 5). However, no significant difference is defined in Figure 9.1, as not all of the sweetcorn cobs examined were grown under identical conditions.

Texture is also a major quality parameter of sweetcorn (Splitter and Shipe 1972; Szymanek *et al.*, 2003) and is influenced by water soluble polysaccharides, tenderness of pericarp and kernel moisture content and turgidity (Culpepper and Magoon, 1927; Bailey and Bailey, 1938; Wann *et al.*, 1971; Szymanek *et al.*, 2003). Heritability is one of the factors influencing quality attributes of sweetcorn including texture. It has been reported that pericarp thickness and soluble solids content depends on genetic variability and that especially in the case of pericarp thickness, heritability is very high (Cardoso *et al.*, 2002). Pericarp thickness has been reported to have a negative relationship with tenderness as assessed by panellists (Bailey and Bailey, 1938). On the other hand, firmness was found to be slightly or insignificantly correlated to kernel tenderness (Azanza *et al.*, 1996a). Several studies have been conducted to assess pericarp tenderness (Bailey and Bailey, 1938; Azanza *et al.*, 1996a; Szymanek *et al.*, 2003). In terms of firmness, sweetcorn kernels have been characterised as ‘firm’ as opposed to ‘mushy’ (Perkins-Veazie *et al.*, 1994). However, kernel firmness has not been studied extensively. Thus, the present study aimed to enhance and elucidate further the sweetcorn textural profile spatially and how this is affected by several storage factors, focusing on kernel firmness. The influence of genotype of kernels firmness is not clearly shown in the experiment described in Chapter 6, when cv. 7210 was compared against cv. Fito 206. However, the same cv. (7210), had significantly lower firmness than cv. 6800 throughout 24 days of storage (Chapter 4), confirming the variability between genotypes.

#### **9.2.4 Storage time as factor influencing sweetcorn quality**

In earlier years, the need for sweetcorn refrigeration for the retention of sugar levels was resolved by the introduction of cvs. with very high initial sugar levels

(Showalter and Miller, 1962). In the current work, it was shown that the storage period negatively affected the total sugar concentration and therefore sweetness. In other work it has also been reported that total soluble solids and sweetness have been found to be reduced over 21 days of storage (Vigneault *et al.*, 2007). However, even at the end of the storage period, sugar content was still very high contributing to the suggestion that, due to the initial high levels of sucrose, sugar content is maintained better than for other types of corn such as standard (*su*) and sugary enhanced corn (*se*).

Storage period had negative effects on the colour parameters (hue angle, lightness and chroma) measured in fresh cv. 7210 only from day 8 to day 10 of the storage period indicating the retention of colour properties during 8 days of storage including lightness and intensity of the colour (Chapter 7). During storage, hue angle values of husks decreased (Chapter 8) in agreement with Geeson *et al.*, (1991) who stated that colour of husks after 10 days of storage at 0.5°C, changed from green to ‘brownish-green’.

After sugars, other chemical compounds of great importance in sweetcorn are those that have antioxidant properties and/or nutritional value such as phenolic compounds. Phenolic compounds are phytochemicals; some of which have reported health promoting potential due to their antioxidant properties as they can scavenge free radicals (Kahkonen *et al.*, 1999; Sun *et al.*, 2002; Atkinson *et al.*, 2005; Lopez-Martinez *et al.*, 2009). Total phenolics are mostly found in the pericarp and aleurone layers of kernels and in lower amounts in the endosperm and thus, are considered to be related with the tissue hardness (Hartley and Ford, 1989). Results for total phenolics were obtained from the experiments described in Chapter 7 and 8. In the experiment described in Chapter 7, total phenolic content declined over the storage period while in the other case (Chapter 8) total phenolic content did not decline. Among the phenolic acids found in corn, ferulic acid is the most abundant phenolic. The effects of storage temperature, time of storage, format of the cobs during storage and cooking was studied in Chapter 8. It was shown that the concentration of ferulic acid reduced after day 4. In addition, L-ascorbic acid content of sweetcorn cobs significantly declined after the first day of storage.

In terms of texture, it is believed that firmness of most fruits and vegetables can be maintained for longer than the postharvest nutrient profile (Kader, 2003). Geeson *et al.*, (1991) in a study that evaluated sweetcorn firmness during storage, stated that

sensory panels believed sweetcorns were ‘slightly firmer’ after storage for 21-24 days. In the present study, firmness fluctuated in an unspecified pattern but generally, decreased at the end of storage period (Chapter 4-8).

### 9.2.5 Effect of storage temperature

Storage temperature is also a very important factor which influences postharvest sweetcorn quality and it has been suggested that the optimum maintenance of quality is achieved when cobs are held as close as possible to 0°C (Brecht, 2002). The biological explanation of the importance of storage temperature on the shelf life of fruits and vegetables relies on the general observation that an increase in temperature of approximately 10°C, results in double or even treble the number of biological reactions occurring in the plant (Riad, 2003). The effect of a temperature change of 10°C on the biological reactions is described as temperature quotient Q<sub>10</sub>. Specifically for sweetcorn, Shiina *et al.* (1997) reported that its respiration rate increases approximately 5-8-fold after an increase in temperature of 20°C, which implies the significance of storage temperature in sweetcorn quality preservation.

In the current PhD the closest temperature to 0°C that was used was 2°C±1. The reason that a storage temperature of 0°C was not tested was that neither at market nor at home is this storage temperature applied. Thus, if 0°C had been used to store the sweetcorn cobs examined, then the findings may have been misleading. Keeping temperature low is crucial as increasing storage temperature results in increased CO<sub>2</sub> and therefore increased sugar utilisation/conversion and water loss (Riad, 2004). Indeed, among the sweetcorn cobs examined, cobs stored at 3°C and 2°C (Chapter 4 and 8 respectively) had higher sugar content than cobs stored at 5°C (Chapter 6 and 7). In addition to storage temperature, controlled atmosphere is also used for better retention of sweetcorn quality. As described in Chapter 4, the combination of 8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub> at 3°C was proved to be an appropriate combination for maintaining sugar content in sweetcorn cobs for 24 days. The second quality attribute of sweetcorn examined extensively in the present work was texture which was affected only occasionally by storage temperature. In fact, when changes in maximum compressive load values were

observed as a result of storage temperature, it was indicated that in lower temperatures, kernels were firmer.

Colour of kernels and husks was influenced by storage temperature (Chapter 8). Colour of kernels and therefore visual quality of the sweetcorns examined, was retained better at 2°C in comparison to 7°C. In more detail, hue angle and lightness were not affected by storage temperature (2 vs. 7°C) but chroma values were lower for cobs stored at 7°C.

Storage temperature affected other quality attributes of sweetcorn. The effects of temperature on total phenolics showed that periodically, cobs stored at 7°C had higher phenolic content than cobs stored at 2°C. Storage temperature at 7°C induced more stress in corn in comparison to 2°C which in turn might cause further production of phenolic compounds (Atkinson *et al.*, 2005). Knowing that at lower temperatures metabolic rates are reduced (Riad, 2004) it was expected that at 7°C, more stress would be induced rather than at a storage temperature of 2°C. For instance, respiration rate, moisture loss and microbial growth is closely related to storage temperature as at higher storage temperature, weight loss, moisture loss, respiration rate and microbial growth is increased. Temperature can also be related to chilling stress or heat stress but this was not an issue in the present study. Generally, at lower temperatures the quality of sweetcorn is better retained and the changes in the biochemical and textural profile of sweetcorn are minimised. At higher temperatures enzymatic and oxidative reactions occur faster (Rodov *et al.*, 1999; Riad and Brecht, 2002; Riad, 2004). Results of ferulic acid content indicated that cobs cooked for 10 min had higher ferulic acid when stored at 7°C, rather than at 2°C. However, this variation was not always reflected in the total phenolics results regarding cobs cooked for 10 min. In contrast, at the storage temperature of 2°C, xanthophyll content was lower than at 7°C while there was no evidence that storage temperature affected L-ascorbic acid content.

#### **9.2.6 Potential relationship between different tissues**

Sugar content of kernels and non-edible tissues of cobs (shank and core), was examined in order to enhance the knowledge about the spatial sugar profiles in

sweetcorn. Szymanek *et al.*, 2003 stated the relatively high concentration of sugars in the sections of the kernels that are closer to the core without explaining adequately the reason of these findings. This finding in addition with the relatively poor literature about sugar profile of the non-edible parts of supersweet cvs., led to the elucidation of the spatial sugar profile in the current study. Yet, no correlation between the tissues examined was identified (Chapter 6). Thus, the present study did not offer a better understanding of the potential interrelationship of sugar content between different tissues. Furthermore, it can be hypothesised that sugar content of one of the type of tissue examined would not be useful as an indicator of the sugar content of another tissue. This is the first piece of work detailing the distribution of individual sugars in core, kernels and shank of supersweet sweetcorn cvs. Total sugar content of kernels, as the sum of glucose, fructose and sucrose was significantly lower than in the core and shank (at 1.21-fold and 1.16-fold lower respectively). Hence, results from the present study enriched information about sugar profile of sweetcorn residues which revealed great nutritional value for feedstock for animals and potential suitability for use as sweeteners. Considering that corn residues are used for the production of energy and that supersweet sweetcorns have greater sugar content than normal corn, it can be assumed that in this case, the core and shank would potentially serve as an even more valuable source of energy for anaerobic digestion. Spatial sugar profile differed among the tissues tested. It was found that shank had higher glucose and fructose content than kernels.

### **9.2.7 Are the spatial changes that occurred in sugar content and texture significant?**

The spatial distribution of sugars between different locations [bottom, middle, top (see Chapter 6)] of kernels and shank was not differentiated, indicating equal sugar quality of all kernels, regardless of their location. Thus industries should not prefer the central parts of the cobs for packs of cobbettes. In turn, the use of good quality kernels located at the top and bottom parts of the cobs for other purposes such as canning processes would be avoided. In contrast, results from the measurement of maximum compressive load, revealed significant spatial variation. In particular, results suggest that

the part of the cobs firstly consumed (central); is firmer than other parts of the cobs which are often removed during processing.

### 9.2.8 Effect of packaging format

The lack of information about the potential influence of the cob format on sugar content of kernels led to the experiments described in Chapters 5, 6 and 8. Results derive from the experiment described in Chapter 6 did not indicate that format of the cobs influenced sugar content of kernels. However, results from the experiment described in Chapter 8 did demonstrate that sugar content of window stripped cobs was higher and better retained than of naked cobs. Therefore, it can be said, that format of the cobs can potentially affect sugar content of sweetcorn cobs. The poor documentation on sugar content comparison between different cob formats is not an adequate argument for the present study. Thus it can be assumed that conversion of sugar to moisture-related starch occurs faster in the absence of husks that prevent dehydration. Additionally, genotype might also play an important role on this as indicated in Section 9.2.3.

Results from other chemical compounds showed that L-ascorbic acid was not significantly differentiated between naked and window stripped cobs while the carotenoid content of sweetcorn cobs stored as naked was higher than in cobs stored as window stripped (Chapter 8). Similarly, raw naked cobs had potentially greater antioxidant capacity and naked cobs had higher ferulic acid content in comparison to window stripped cobs. The main reason that packaging format can affect quality attributes of sweetcorn is probably the higher moisture content that window stripped cobs have, in comparison to naked cobs (Chapter 5, 6 and 8). A strong correlation between moisture and sugar levels of kernels has been reported while moisture content has also been found to be related with kernel texture (Azanza *et al.*, 1996b; Karababa and Coscuner, 2007). In particular, the high levels of sugars lead to an osmotic retention of moisture (Ferguson *et al.*, 1979) which explains the positive relationship between sugars and moisture content. In addition to this observation, it has been found that in decreased moisture content, the rate of conversion of sugars to starch is higher (Douglass and Juvik, 1993), and therefore the positive relationship between moisture content and sugars is again implied. Furthermore, the higher moisture content in the basal location of the kernels closer to the



cobs, compared to the moisture content of the upper part of the kernels (Szymanek *et al.*, 2003) implies that the greater surface of the pericarp in the outer part of the kernels has different texture and biochemical composition in terms of sugars and starch, compared to the smaller volume of the basal endosperm that has lower moisture content. In addition, considering the greater amount of phenolic compounds in the pericarp rather than in the endosperm, it also explains that the lower moisture content (of naked cobs compared to window stripped cobs) indicates greater phenolic content. Antioxidant compounds in the edible parts of sweetcorn are mostly stored in the pericarp (Riad, 2003). On the other hand, deterioration occurs faster in naked cobs in comparison to window stripped cobs probably due to faster dehydration. This deterioration might lead to greater release of antioxidant compounds which might explain why naked cobs appear to have greater potential of antioxidant properties than window stripped cobs. Hence, this study contributes to the awareness of the potential antioxidant profile of sweetcorn as affected by the existence of husks during storage and suggests that when purchase is decided under criteria related to potential healthfulness rather than flavour, naked cobs might be more beneficial.

Format of the cobs in terms of length of shank was also examined. Usually the shank of the cobs is trimmed before packaging to *ca.* 2-4 cm otherwise it is believed to cause reduction of moisture content and practical problems during packaging (Turk *et al.*, 2001; Szymanek *et al.*, 2003). In the current study, cobs stored with short (2 cm) and long (8 cm) shanks were tested for their sugar content during storage. There was no significant interaction between length of shank and storage period. Thus, the advice to packers is to promote cobs with short shanks for reduction of space and minimisation of financial cost.

Periodically, firmness of the cobs examined was affected by the absence of husks (Chapter 5, 6 and 8). In all cases, naked cobs proved to be firmer than window stripped cobs, while results derived from cobs stored with different length of shanks were inconsistent. The variation between texture of naked and window stripped cobs is related to the differences between their moisture content as previously explained.

There is a lack of information regarding colour variations between naked and window stripped cobs over long-term storage. However, colour (hue angle) is considered to be linked with carotenoids (Fanning *et al.*, 2010). In the present study, hue angle and chroma were also affected by the format of the cobs, suggesting that window stripped

cobs had higher hue angle values and intensity of colour (chroma) in comparison to naked ones and therefore would probably be preferred. Variation in colour parameters can be explained by carotenoid content as correlations between these parameters were statistically significant, yet not as strong as expected. Nevertheless, it is speculated, that colour parameters measured were greater in window stripped cobs due to the protective shield of husks towards factors affecting colour such as stress-induced compounds.

## **9.2.9 Effect of cooking on nutritional quality**

### **9.2.9.1 Effect of cooking on quality attributes of sweetcorn kernels**

Sweetcorn is usually consumed after it has been cooked and therefore the elucidation of sugar content alterations after cooking is essential. In the present study, the cooking method used was a ‘home-style’ method of cooking, using a microwave. According to Degner *et al.* (2001), boiling is the most common way of cooking at home even if it is a time-consuming method, in contrast to microwave cooking. However, it has been suggested that nutritional loss of foods in the microwave is lower or equal compared to other methods of cooking (Lorenz, 1976). Generally the more the vegetables are cooked, the higher is their nutritional loss (Makhlouf *et al.*, 1995) and the effects of cooking depend on the vegetable (Rickman *et al.*, 2007b). Regarding sweetcorn, it has been found that sugar content decreases with boiling (Riad *et al.*, 2003). Generally, in both experiments (Chapter 7 and 8), total sugar loss after cooking sweetcorn cobs was examined. The significant differences observed were not as high as expected, indicating that consumer satisfaction in terms of sugars and therefore taste would not be affected by commonly applied cooking times in the microwave.

Cooking had significant effects on the colour parameters measured. In particular, hue angle increased after cooking probably as a result of the upregulation of polyphenolic compounds (Perkins-Veazie 1994; Riad, 2004). However, no correlation between such compounds and hue angle was found. Furthermore, there was no evidence that colour was also affected by different cooking times (5 min vs. 10 min). In contrast, firmness (as estimated by maximum compressive load), decreased as cooking times increased in agreement with Bourne (1982) who stated the negative relationship between temperature

and firmness (see Section 7.3.3 and Section 8.3.4). Additionally, results from the experiment described in Chapter 8, indicate the variation of firmness between both sweetcorn cobs stored as naked and window stripped and between sweetcorn cobs stored under different format at different temperatures (2°C and 7°C). To highlight the results of Figure 8.10, the greater firmness of naked cobs and cobs stored at 2°C was shown when compared to window stripped cobs and cobs stored at 7°C, respectively. Tissue softening upon cooking has been explained in Section 2.5.1 through the negative relationship between temperature and firmness. On the other hand, the greater firmness observed in absence of husks and in storage temperature has been explained through loss of moisture content (see Section 8.3.4). The effects of format, cooking time and storage temperature were also shown and as interaction of these factors (Chapter 8).

Generally a loss in total phenolic content after cooking was observed but not between cobs cooked for different times. It was therefore implied that the comparison of conventional cooking protocols differing in times does not affect significantly total phenolic content. In a previous study, it has also been suggested that total phenolic content of fresh-cut sweetcorn kernels stored at 5°C was not affected by storage time but was affected negatively by cooking (Riad *et al.*, 2003). However, authors did not correlate the variants examined to elucidate the relationship between them. Cobs cooked for 10 min had higher ferulic content than cobs cooked for 5 min and uncooked cobs. The variation observed in ferulic acid content can probably be explained by a combination of conditions. Ferulic acid's role in creating covalent bonds with polysaccharides, the existence of free radicals and the release of ferulic acid by ester glycosides found in the cell-walls might contribute to these variations.

Cooking treatments were detrimental to L-ascorbic acid content which was expected according to previous studies (Dewanto *et al.*, 2002). Decrease of ascorbic acid can be explained by its oxidation to other compounds such as dehydroascorbic acid and polymerization that leads to the production of compounds that do not act as vitamin C (Gregory, 1996).

The highest amount of L-ascorbic acid quantified in the cobs examined was *ca.* 5mg/100g on a fresh weight basis. It is worth noting, that the recommended daily intake for vitamin C in adults is 40 mg [COMA, 1991; UK Food Standards Agency, 2002]. Therefore, at its peak point, one portion of the sweetcorn examined would reach 10% of

the previously mentioned recommended dose. Okiei *et al.* (2009) in an effort to compare titrimetric and voltametric methods for the assessment of L-ascorbic acid analysed several fruits and vegetables. The authors found that sweetcorn and other commonly consumed commodities in the UK such as bananas and apples do not have high concentrations of ascorbic acid in contrast to peppers, oranges, cherries and other products that exceeded the 50mg/100g of ascorbic acid. When considering evidence for the 'five a day message', the World Health Organization found that the risk of development of several diseases is reduced when 400 g of various fruits and vegetables was consumed. Thus, this message suggests the daily consumption of 5 portions (80 g each) of fruits and vegetables. It can therefore be said that 'five a day' portion of sweetcorn, would minimally contribute to the suggested daily intake of vitamin C. However, this can be confirmed after quantification of the other active form of vitamin C- dehydroascorbic acid.

#### **9.2.9.2 Carotenoid content and stability after cooking**

Compounds with antioxidant properties in corn are also the carotenoids that potentially have beneficial effects against degenerative diseases (Kopsell and Kopsell, 2006). Carotenoids also have protective properties against the natural process of oxidation which is inevitable and might potentially lead to several diseases (Roberfroid, 1995). In sweetcorn carotenoids are found in kernels. In the review by Kopsell and Kopsell (2006) carotenoid concentration was said to be dependent on the variety, the physiological characteristics and the presence and concentration of other chemical compounds found in sweetcorn. Information about the variation in carotenoid content can be used in breeding programmes in order to produce sweetcorn with enhanced concentration of carotenoids. The most commonly found carotenoids in sweetcorn are lutein and zeaxanthin, while  $\beta$ -carotene and  $\beta$ -cryptoxanthin occur at very low levels (Kurilich and Juvik, 1999b; Scott and Eldridge, 2005; Howe and Tanumihardjo, 2006). In the current study it was also found that lutein and zeaxanthin were detectable and quantifiable in contrast to  $\beta$ -cryptoxanthin and  $\beta$ -carotene which were occasionally detectable but could not be quantified. Lutein and zeaxanthin belong to the sub-category of xanthophylls and are associated with age-related macular degeneration (Seddon *et al.*,

1994). In addition to sugar content, it has been reported that carotenoid content in sweetcorn cobs including the *sh2* gene, is higher than in standard and sugary enhanced sweetcorn (Kurilich and Juvik, 1999) and probably in yellow sweetcorn higher than other colour-types of corn (Kopsell *et al.*, 2009). Considering that xanthophyll content of the examined cobs was *ca.* 0.02mg/g, they can be considered to be highly pigmented (Holden *et al.*, 1999; Scott and Eldridge, 2005).

In absence of colour and carotenoid changes, it can be hypothesised that cooking did not affect carotenoid content. Hart and Scott (1995), in a report where several vegetables were studied, stated that cooking minimally affected carotenoid content. It is worth noting that the same authors, included fresh and cooked sweetcorn in their research and hypothesized that the bioavailability of carotenoids are higher in cooked products rather than their fresh commodities as cooking might boost their extractability. In the present work, comparison of uncooked vs. cooked kernels showed that cooked kernels had decreased concentration of xanthophylls (Chapter 8). It was thus implied, that even if the extractability of carotenoids was boosted upon cooking, this was outweighed by the susceptibility of carotenoid chemistry to heat treatments. .

### 9.3 Project conclusions

The objectives of the project were presented in Chapter 1, Section 1.2.2. Conclusions of the project are briefly summarised below:

- *To contribute to method development, validation and optimisation of textural characteristics and extraction and quantification of target analytes related to major quality parameters.* Texture was assessed by measuring maximum compressive load values and can be considered as an adequate and appropriate method for the evaluation of kernel firmness. Total sugars were identified and quantified by optimised and validated HPLC procedures that are considered to be valid especially in comparison to the commonly used measurements of total soluble solids. In addition, high sugar values indicate that the method used, might be better than existing published HPLC methods on maize. Estimation of starch values, matched with the expected values according to the literature and therefore the method used can be considered suitable for starch

quantification. The methods used for the quantification of total phenolic content and especially of total antioxidant activity (Sections 3.11.1 and 3.13 respectively) were not sufficiently accurate. The methods used for the measurement of ferulic acid, L-ascorbic acid and xanthophylls were developed, optimised, validated and demonstrated as being suitable.

- To investigate the influence of the genotype and of the origin of the cobs on texture and sugar content of sweetcorn cobs containing the *sh2* gene. Genotype and origin of the cobs had significant influence in the texture and sugar content of the sweetcorn cobs examined (Chapter 4, 5 and 6).
- To determine potential differences in the texture and the concentrations of sucrose, glucose and fructose in cobs of sweetcorn cultivars (cvs.) stored at different temperatures. Sucrose was the predominant sugar in all samples tested regardless of storage temperature (Chapter 5-8). However, lower storage temperatures retain better sugar content of cobs tested. Thus, the best storage temperature tested in this thesis was 2°C in terms of preservation of the quality attributes examined.
- To determine differences in texture-related characteristics of sweetcorn cvs. of different format; such as sweetcorn cobs covered with husks vs. cobs without husks. It was indicated that naked cobs were firmer than window stripped cobs (Chapter 5, 6 and 8).
- To investigate the spatial, textural and sugar content changes throughout sweetcorn cobs. Sugar profile of the tissues studied was different. Sugar content of kernels was lower than of shank and core. Spatially (bottom, middle and top of the cobs) there were not any significant differences in the sugar profile and content of kernels. In terms of texture, kernels located in the central parts of the cobs were firmer.
- To elucidate the colour and the nutritional and textural changes occurring under cooking conditions when stored at different temperatures and in different cob formats (with or without husks). Generally, the antioxidant compounds analysed in the sweetcorn kernels tested were negatively affected by cooking but not according to cooking time

(Chapter 8). Firmness of kernels reduced over cooking time (Chapter 7 and 8). The presence of husks in the cobs during storage was periodically found to influence the texture and nutritional attributes of the sweetcorn cobs tested (Chapter 8).

#### **9.4 Recommendations for future work**

Sugar losses, reduction of moisture content and increased firmness are major causes of decreased sweetcorn quality over storage period. Different storage conditions might lead to different levels of quality. Thus, format of the cobs at storage, requires further research in order to elucidate the procedures and the conditions which can influence quality attributes of interest. Combination of the appropriate temperature and modified atmosphere that would allow transfer of gases in such a way that would maintain CO<sub>2</sub> and O<sub>2</sub> in the concentrations studied in Chapter 4 (8 kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>), would also lead to valuable conclusions and would potentially have beneficial applications in industry. Hence, further investigation on the effects of the CA atmosphere used in addition to analytes of interest and comparison with other CA conditions, is required.

Ratings of quality from sensory panels would also be helpful in better confirming the perception of consumers about differences caused by different storage conditions, genotypes and any other source of variation. However, the experimental design of such work would be quite difficult due to the enormous size of experiment. The potential relationship between total sugar content of core, shank and kernels is possibly of great interest as it would lead to important conclusions and new strategies for the retention of sugars, e.g. factors that would influence the transport of sugars from the non-edible parts of the cobs. Breeding programmes for sweetcorn mainly involved sucrose accumulation and starch deficient mutants while existence of a relationship between different tissues would probably lead to new breeding programmes targeting the desirable properties of the non-edible tissues of corn.

Further investigation is also required on the concentration of antioxidant compounds found in sweetcorn as affected by postharvest factors. It is not fully clear yet under which circumstances total antioxidant activity changes and in order to be elucidated, more than one method for the measurement of total antioxidant activity

should be employed. Except for the chemical assays commonly used for the measurement of total antioxidant capacity; cellular antioxidant activity (CAA) has been recently suggested to be an appropriate biological method for evaluation of total antioxidant activity in several fruits and vegetables including sweetcorn (Song *et al.*, 2010). Further evaluation of total antioxidant activity as affected by the factors investigated, would also be worth carrying out due to the significant correlation of CAA values with total phenolic content. This recommendation relies on the incapability of a unique method to quantify with accuracy the total antioxidant activity, as a sum of the antioxidant activities of individual compounds that react differently to each method currently used. However, even accurate measurements of total antioxidant capacity should be combined with a deeper knowledge of the bioavailability. Hence, bioavailability of individual antioxidant compounds contained in supersweet sweetcorn cvs. is required in order to conclude valid and profitable information for consumers regarding the protection against several diseases. Furthermore, dietary guidelines could be enhanced and made more accurate. It is worth noting that in some cases such as L-ascorbic acid, ferulic acid and carotenoids, bioavailability is easily altered as a result of postharvest factors influencing postharvest quality by causing changes in the chemistry of such compounds.

The comparison of alterations occurring in texture and chemical composition of sweetcorn under different cooking conditions in terms of cooking equipment is essential. In more detail, the small number of alterations which occurred between sweetcorn cobs cooked for different times in the microwave generates questions that could probably be answered through further research followed by a comparison between other methods and/or other additional analysis of components related to the variants measured in the current study. The rate that microwave cooking affects nutrition of sweetcorn during postharvest life should also be further investigated. Moreover, changes in quality parameters of sweetcorn and factors affecting them would be useful if evaluated simultaneously. Further investigation is also required for the potential correlation between the most abundant yellow pigments found in sweetcorn (xanthophylls) and colour properties (hue angle, lightness and chroma). Then the promotion of sweetcorn with enhanced concentration of these antioxidant compounds would be facilitated, encouraging consumption of sweetcorn combining unique colour with healthfulness.



Possible implications of the results found in the present study could potentially lead to new applications in industry but in order to detail them; factors such as spatial textural profile of sweetcorn regarding the increased firmness of kernels located in the central parts of the cobs (Chapter 6) should be further studied. Apart from applications in industry, these results could probably affect consumer preferences by increasing their perception of *sh2*-sweetcorn as a source of nutritionally valuable attributes combining satisfactory flavour.

## 10. CHAPTER TEN

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## **11. CHAPTER ELEVEN**

### **Appendices**

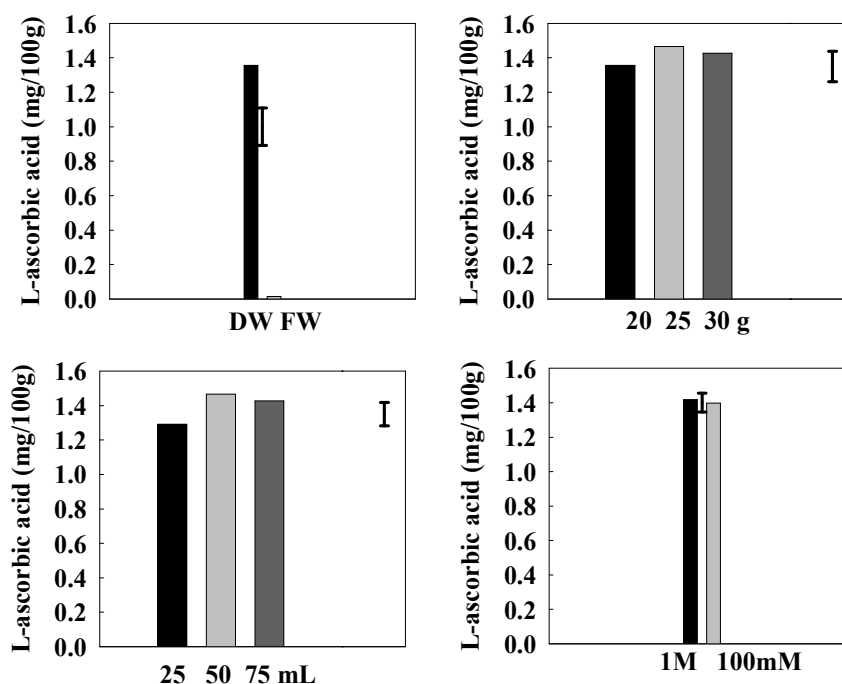
#### **APPENDIX A**

##### **Trials for the measurements of organic acids**

###### **A.1 L-ascorbic acid assay: optimisation of the analysis method**

For the analysis of L-ascorbic acid, different extraction methods based on the protocol of Megazyme ltd were examined (see Section 3.10.2). In more detail, samples were also tested for their L-ascorbic acid concentration starting with different amounts of fresh sample (20, 25 and 30 g), different volumes of potassium phosphate buffer, different molarities of the buffer (1 M) and different format of the initial sample (freeze-dried vs. frozen) for the extraction of samples. For the trials, five samples for each treatment were used. Results are presented in Figure A.1.

Results indicated that freeze-dried samples were not appropriate for L-ascorbic acid analysis. When frozen samples for the analysis of L-ascorbic acid were used, insignificant differences between extracts of 20 mg, 25 mg and 30 g of kernels were observed. The volume of potassium phosphate buffer resulted in significantly inferior L-ascorbic acid concentration in extracts when 25 mL were used. The molarity of potassium phosphate buffer was not proved to be significant source of variation for L-ascorbic acid in the samples tested.

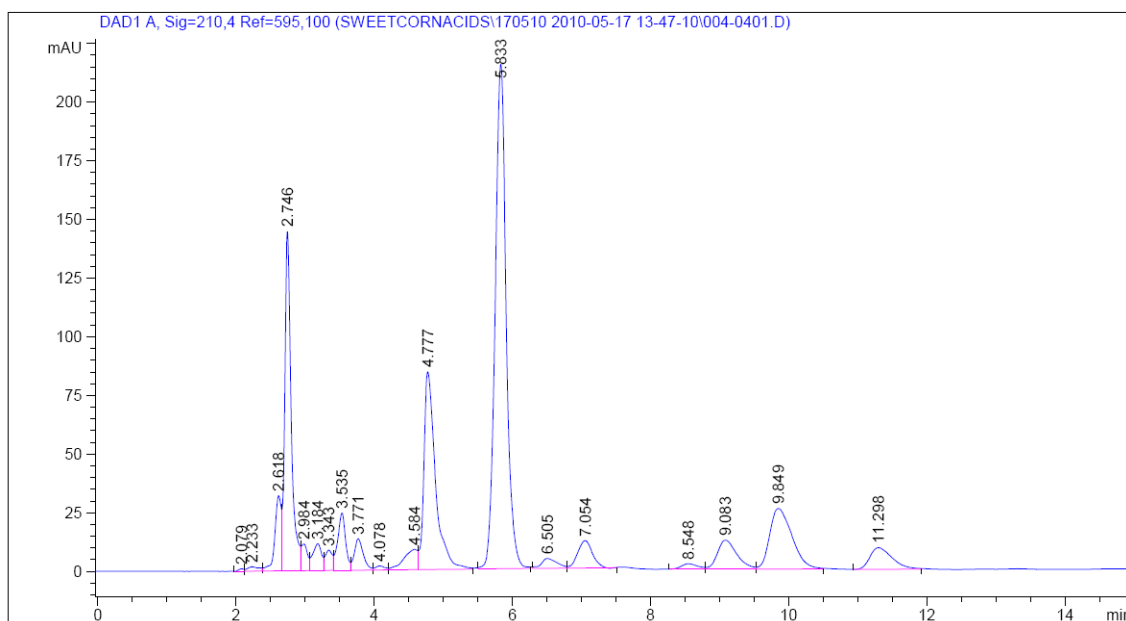


**Figure A.1:** Comparison of L-ascorbic acid concentration of: a) frozen samples (FW) vs. freeze-dried samples (DW), b) samples extracted using 20 mg vs. 25 mg vs. 30 mg, of kernels (FW); c) samples extracted using 25 mL, vs. 50 mL vs. 75 mL of potassium phosphate buffer and d) samples extracted using 1M vs. 100mM of phosphate buffer.

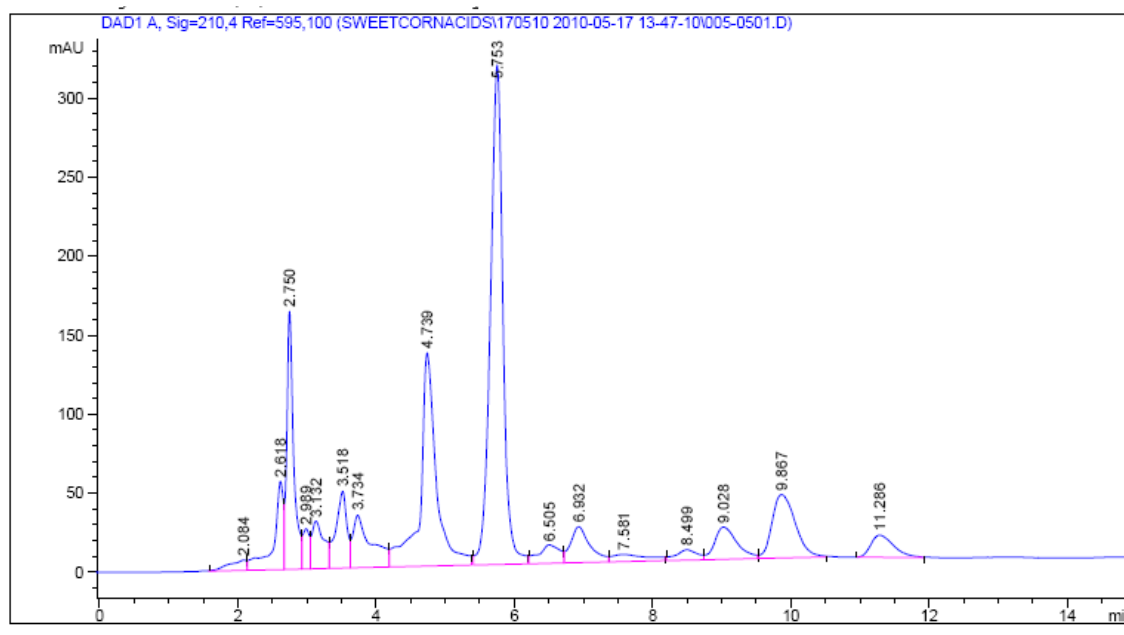
## A.2 Methods tested for identification of organic acids

Different quantities of lyophilised sweetcorn samples, were used and compared for identification and quantification of ascorbic acid (see Section 3.10.1). Representative chromatograms are presented below.

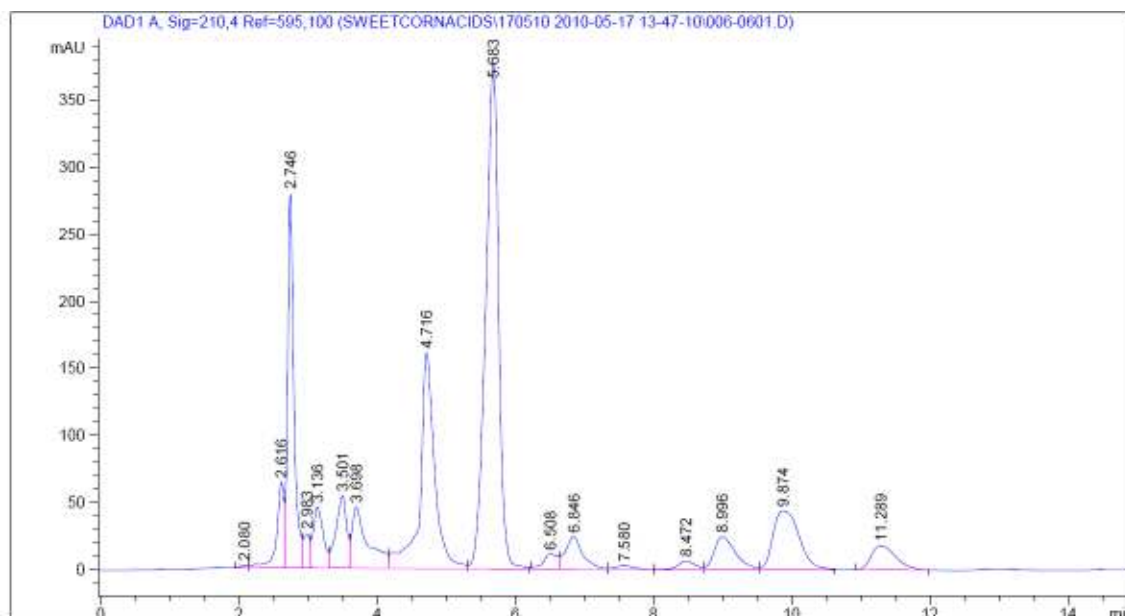




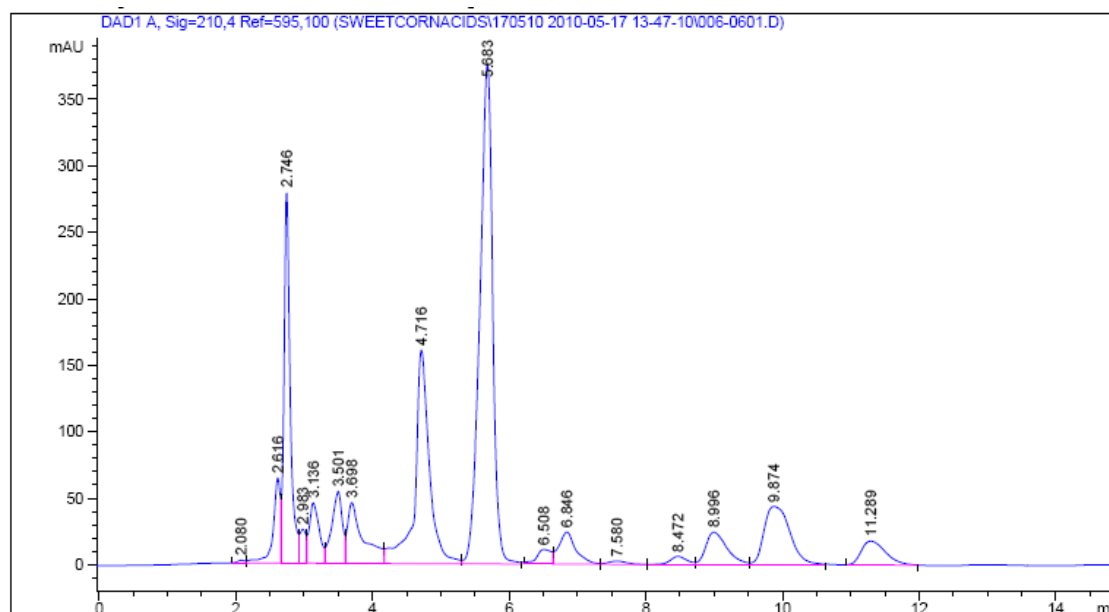
**Figure A.2:** Representative chromatogram obtained from the analysis of organic acids from sweetcorn extracts (initial weight of kernels used: 100mg).



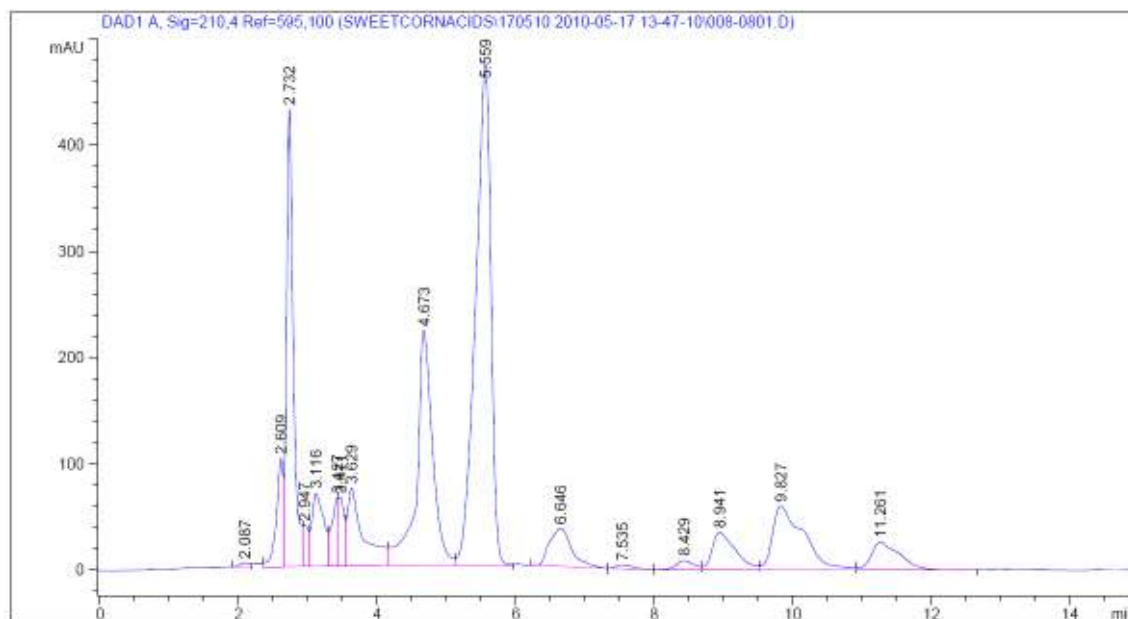
**Figure A.3:** Representative chromatogram obtained from the analysis of organic acids from sweetcorn extracts (initial weight of kernels used: 150mg).



**Figure A.4:** Representative chromatogram obtained from the analysis of organic acids from sweetcorn extracts (initial weight of kernels used: 200mg).



**Figure A.5:** Representative chromatogram obtained from the analysis of organic acids from sweetcorn extracts (initial weight of kernels used: 250mg).



**Figure A.6:** Representative chromatogram obtained from the analysis of organic acids from sweetcorn extracts (initial weight of kernels used: 300mg).

## APPENDIX B

### STATISTIC TABLES

#### ANOVA tables

**Table B.1** Kernel sucrose concentration on dry weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	184165.00	30694.00	22.69	<.001
Cultivar	6	309238.00	51540.00	38.09	<.001
Storage time. Cultivar	36	135315.00	3759.00	2.78	<.001
Residual	193	261115.00	1353.00		
Total	241	886883.00			

**Table B.2** Kernel glucose concentration on dry weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	4093.59	682.27	8.23	<.001
Cultivar	6	4243.02	707.17	8.53	<.001
Storage time. Cultivar	36	3259.98	90.56	1.09	0.343
Residual	193	16007.68	82.94		
Total	241	27457.53			

**Table B.3** Kernel fructose concentration on dry weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	572.40	95.40	1.79	0.103
Cultivar	6	2895.57	482.59	9.05	<.001
Storage time. Cultivar	36	2295.49	63.76	1.20	0.222
Residual	193	10294.86	53.34		
Total	241	16018.41			

**Table B.4** Kernel total sugar concentration on dry weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	205459.00	34243.00	19.22	<.001
Cultivar	6	375294.00	62549.00	35.11	<.001
Storage time. Cultivar	36	163957.00	4554.00	2.56	<.001
Residual	193	343819.00	1781.00		
Total	241	1085832.00			

**Table B.5** Kernel sucrose concentration on fresh weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	10555.40	1759.20	13.24	<.001
Cultivar	6	35219.40	5869.90	44.17	<.001
Storage time. Cultivar	36	7564.60	210.10	1.58	0.027
Residual	193	25646.90	132.90		
Total	241	78518.00			

**Table B.6** Kernel glucose concentration on fresh weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	240.18	40.03	7.23	<.001
Cultivar	6	263.63	43.94	7.94	<.001
Storage time. Cultivar	36	189.64	5.27	0.95	0.552
Residual	193	1067.92	5.53		
Total	241	1755.22			

**Table B.7** Kernel fructose concentration on fresh weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	33.63	5.61	1.73	0.115
Cultivar	6	54.11	9.02	2.79	0.013
Storage time. Cultivar	36	124.40	3.46	1.07	0.374
Residual	193	623.77	3.23		
Total	241	835.67			

**Table B.8** Kernel total sugar concentration on fresh weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Storage time	6	11707.40	1951.20	10.47	<.001
Cultivar	6	42393.50	7065.60	37.90	<.001
Storage time. Cultivar	36	9303.60	258.40	1.39	0.085
Residual	193	35980.40	186.40		
Total	241	98886.80			

**Table B.9** Kernel sweetness on fresh weight basis (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	6	71229.70	11871.60	61.53	<.001
Storage time	5	28299.70	5659.90	29.33	<.001
Storage time.Cultivar	30	9233.20	307.80	1.60	0.035
Residual	163	31450.70	192.90		
Total	204	138564.00			

**Table B.10** Maximum compressive load (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	6	495365.00	82561.00	43.48	<.001
Day	6	130271.00	21712.00	11.43	<.001
Cultivar.Day	34	144784.00	4258.00	2.24	<.001
Residual	171	324730.00	1899.00		
Total	217	823036.00			

**Table B.11** Kernel moisture content (Chapter 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	6	1139.78	189.96	34.82	<.001
Storage time	5	95.92	19.18	3.52	0.005
Cultivar.Storage time	30	199.80	6.66	1.22	0.215
Residual	166	905.60	5.46		
Total	207	2324.67			

**Table B.12** Sucrose DW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	25615.	8538.	7.04	<.001
Residual	34	41255.	1213.		
Total	37	66870.			

**Table B.13** Glucose DW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	747.58	249.19	13.03	<.001
Residual	34	650.05	19.12		
Total	37	1397.63			

**Table B.14** Fructose DW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	309.39	103.13	9.81	<.001
Residual	34	357.49	10.51		
Total	37	666.88			

**Table B.15** Total sugars DW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	30470.	10157.	6.83	0.001
Residual	34	50550.	1487.		
Total	37	81021.			

**Table B.16** Sucrose FW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	1657.55	552.52	8.23	<.001
Residual	34	2282.32	67.13		
Total	37	3939.86			

**Table B.17** Glucose FW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	38.0253	12.6751	17.11	<.001
Residual	34	25.1825	0.7407		
Total	37	63.2078			

**Table B.18** Fructose FW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	11.5240	3.8413	11.23	<.001
Residual	34	11.6275	0.3420		
Total	37	23.1515			

**Table B.19** Total sugars FW (Chapter 5-Experiment 2)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar-packaging	3	2005.75	668.58	8.40	<.001
Residual	34	2706.05	79.59		
Total	37	4711.80			

**Table B.20** CO<sub>2</sub> concentration (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	6	1104.18	184.03	17.55	<.001
Format	1	226.51	226.51	21.60	<.001
Origin	1	142.96	142.96	13.63	<.001
Temperature	1	3341.45	3341.45	318.68	<.001
Day.Format	6	117.40	19.57	1.87	0.091
Day.Origin	6	57.08	9.51	0.91	0.492
Format.Origin	1	12.87	12.87	1.23	0.270
Day.Temperature	4	760.32	190.08	18.13	<.001
Format.Temperature	1	109.03	109.03	10.40	0.002
Origin.Temperature	1	65.42	65.42	6.24	0.014



**Table B.20** CO<sub>2</sub> concentration (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format.Origin	6	34.97	5.83	0.56	0.765
Day.Format.Temperature	4	34.96	8.74	0.83	0.506
Day.Origin.Temperature	4	21.10	5.27	0.50	0.734
Format.Origin.Temperature	1	26.30	26.30	2.51	0.115
Residual	142	1488.92	10.49		
Total	185	6564.51			

**Table B.21** O<sub>2</sub> (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	3	55.989	18.663	3.61	0.018
Format	1	12.236	12.236	2.36	0.129
Origin	1	0.451	0.451	0.09	0.769
Temperature	1	935.859	935.859	180.82	<.001
Day.Format	3	9.662	3.221	0.62	0.603
Day.Origin	3	23.586	7.862	1.52	0.218
Format.Origin	1	0.021	0.021	0.00	0.949
Day.Temperature	1	92.665	92.665	17.90	<.001
Format.Temperature	1	11.279	11.279	2.18	0.145
Origin.Temperature	1	6.051	6.051	1.17	0.284
Day.Format.Origin	3	1.163	0.388	0.07	0.973
Day.Format.Temperature	1	4.503	4.503	0.87	0.354
Day.Origin.Temperature	1	8.492	8.492	1.64	0.205
Format.Origin.Temperature	1	3.703	3.703	0.72	0.401
Residual	65	336.425	5.176		
Total	87	1185.058			

**Table B.22** Sucrose DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	1867.7	1867.7	2.30	0.137
Format	1	541.5	541.5	0.67	0.419

**Table B.22** Sucrose DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Origin	1	168040.7	168040.7	206.51	<.001
Temperature	1	2151.1	2151.1	2.64	0.111
Day.Format	1	2172.4	2172.4	2.67	0.109
Day.Origin	1	3624.8	3624.8	4.45	0.041
Format.Origin	1	3843.7	3843.7	4.72	0.035
Day.Temperature	1	2301.2	2301.2	2.83	0.100
Format.Temperature	1	55.0	55.0	0.07	0.796
Origin.Temperature	1	236.0	236.0	0.29	0.593
Day.Format.Origin	1	160.1	160.1	0.20	0.660
Day.Format.Temperature	1	33.9	33.9	0.04	0.839
Day.Origin.Temperature	1	287.4	287.4	0.35	0.555
Format.Origin.Temperature	1	426.4	426.4	0.52	0.473
Residual	44	35803.8	813.7		
Total	58	203374.0			

**Table B. 23** Glucose DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	0.00	0.00	0.00	0.995
Format	1	16.29	16.29	0.58	0.450
Origin	1	2650.98	2650.98	94.65	<.001
Temperature	1	2.97	2.97	0.11	0.746
Day. Format	1	28.76	28.76	1.03	0.316
Day. Origin	1	5.62	5.62	0.20	0.656
Format.Origin	1	153.57	153.57	5.48	0.024
Day.Temperature	1	3.23	3.23	0.12	0.736
Format.Temperature	1	0.05	0.05	0.00	0.968
Origin.Temperature	1	0.28	0.28	0.01	0.921
Day.Format. Origin	1	2.04	2.04	0.07	0.789
Day.Format.Temperature	1	0.02	0.02	0.00	0.979
Day.Origin.Temperature	1	0.36	0.36	0.01	0.911
Format.Origin.Temperature	1	0.86	0.86	0.03	0.862

**Table B. 23** Glucose DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Residual	44	1232.42	28.01		
Total	58	3811.98			

**Table B.24** Fructose DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	82.95	82.95	2.98	0.091
Format	1	77.80	77.80	2.79	0.102
Origin	1	509.51	509.51	18.29	<.001
Temperature	1	15.30	15.30	0.55	0.462
Day.Format	1	3.61	3.61	0.13	0.721
Day.Origin	1	43.68	43.68	1.57	0.217
Format.Origin	1	83.81	83.81	3.01	0.090
Day.Temperature	1	13.72	13.72	0.49	0.486
Format.Temperature	1	0.78	0.78	0.03	0.868
Origin.Temperature	1	6.94	6.94	0.25	0.620
Day.Format.Origin	1	93.10	93.10	3.34	0.074
Day.Format.Temperature	1	1.19	1.19	0.04	0.838
Day.Origin.Temperature	1	5.89	5.89	0.21	0.648
Format.Origin.Temperature	1	7.27	7.27	0.26	0.612
Residual	44	1225.70	27.86		
Total	58	2005.39			

**Table B.25** Total sugars DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	1	2735.	2735.	2.48	0.122
Format	1	1305.	1305.	1.19	0.282
Origin	1	234244.	234244.	212.82	<.001
Temperature	1	2706.	2706.	2.46	0.124
Day.Format	1	2507.	2507.	2.28	0.138
Day.Origin	1	3132.	3132.	2.85	0.099

**Table B.25** Total sugars DW (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Format.Origin	1	6980.	6980.	6.34	0.016
Day.Temperature	1	2859.	2859.	2.60	0.114
Format.Temperature	1	72.	72.	0.07	0.799
Origin.Temperature	1	343.	343.	0.31	0.579
Day.Format. Origin	1	563.	563.	0.51	0.478
Day.Format. Temperature	1	50.	50.	0.05	0.833
Day.Origin. Temperature	1	399.	399.	0.36	0.550
Format.Origin. Temperature	1	356.	356.	0.32	0.572
Residual	44	48429.	1101.		
Total	58	280477.			

**Table B.26** Maximum compressive load (Chapter 5-Experiment 3)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	6	318.875	53.146	34.20	<.001
Format	1	186.796	186.796	120.22	<.001
Origin	1	1050.100	1050.100	675.84	<.001
Temperature	1	0.231	0.231	0.15	0.700
Day.Format	6	141.250	23.542	15.15	<.001
Day.Origin	6	154.305	25.718	16.55	<.001
Format.Origin	1	20.974	20.974	13.50	<.001
Day.Temperature	4	6.357	1.589	1.02	0.394
Format.Temperature	1	7.720	7.720	4.97	0.026
Origin.Temperature	1	36.611	36.611	23.56	<.001
Day.Format.Origin	5	102.079	20.416	13.14	<.001
Day.Format. Temperature	4	57.286	14.322	9.22	<.001
Day.Origin. Temperature	4	45.706	11.427	7.35	<.001
Format.Origin. Temperature	1	0.655	0.655	0.42	0.516
Residual	2009	3121.515	1.554		
Total	2051	4475.122			

**Table B.27** Moisture content (Chapter 6, Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Format	1	3.352	3.352	0.41	0.520
Days	5	286.590	57.318	7.08	<.001
Tissue	2	12625.593	6312.796	779.50	<.001
Length of shank	1	2.377	2.377	0.29	0.588
Format.Days	5	43.110	8.622	1.06	0.380
Format.Tissue	2	10.054	5.027	0.62	0.538
Days.Tissue	10	110.565	11.057	1.37	0.195
Format. Length of shank	1	6.413	6.413	0.79	0.374
Days. Length of shank	5	168.640	33.728	4.16	0.001
part. Length of shank	2	7.821	3.910	0.48	0.617
Format. Days. Tissue	9	47.097	5.233	0.65	0.757
Format. Days. Length of shank	5	47.303	9.461	1.17	0.325
Format. Tissue. Length of shank	2	62.479	31.239	3.86	0.022
Days. Tissue. Length of shank	9	102.387	11.376	1.40	0.185
Residual	321	2599.639	8.099		
Total	380	15182.222			

**Table B.28** Sucrose concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	35544.	7109.	2.58	0.028
Format	1	48.	48.	0.02	0.895
Length of shank	1	3084.	3084.	1.12	0.291
Tissue	2	723030.	361515.	131.23	<.001
Day.Format	5	10555.	2111.	0.77	0.575
Day.Length of shank	5	12764.	2553.	0.93	0.465
Format.Length of shank	1	4996.	4996.	1.81	0.180
Day.Tissue	10	86888.	8689.	3.15	<.001
Format.Tissue	2	6144.	3072.	1.12	0.330
Length of shank.Tissue	2	6936.	3468.	1.26	0.286
Day.Format.Length of shank	5	16447.	3289.	1.19	0.314

**Table B.28** Sucrose concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format.Tissue	10	33000.	3300.	1.20	0.294
Day.Length of shank.Tissue	10	29518.	2952.	1.07	0.386
Format.Length of shank.Tissue	2	2968.	1484.	0.54	0.584
Residual	195	537204.	2755.		
Total	256	1447272.			

**Table B.29** Glucose concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	16052.0	3210.4	5.51	<.001
Format	1	1016.6	1016.6	1.74	0.188
Length of shank	1	52.2	52.2	0.09	0.765
Tissue	2	423729.5	211864.7	363.56	<.001
Day.Format	5	3853.9	770.8	1.32	0.256
Day.Length of shank	5	11145.4	2229.1	3.83	0.003
Format.Length of shank	1	2203.4	2203.4	3.78	0.053
Day.Tissue	10	29329.8	2933.0	5.03	<.001
Format.Tissue	2	6381.9	3190.9	5.48	0.005
Length of shank.Tissue	2	180.6	90.3	0.15	0.857
Day.Format.Length of shank	5	3060.1	612.0	1.05	0.389
Day.Format.Tissue	10	13286.1	1328.6	2.28	0.015
Day.Length of shank.Tissue	10	3195.0	319.5	0.55	0.854
Format.Length of shank.Tissue	2	1153.3	576.6	0.99	0.374
Residual	195	113636.5	582.8		
Total	256	580158.4			

**Table B.30** Fructose concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	26002.9	5200.6	8.78	<.001
Format	1	1954.0	1954.0	3.30	0.071
Length of shank	1	375.3	375.3	0.63	0.427
Tissue	2	313375.1	156687.6	264.59	<.001
Day.Format	5	370.4	74.1	0.13	0.987
Day.Length of shank	5	8913.2	1782.6	3.01	0.012
Format.Length of shank	1	2666.8	2666.8	4.50	0.035
Day.Tissue	10	26639.4	2663.9	4.50	<.001
Format.Tissue	2	2639.7	1319.8	2.23	0.110
length.Tissue	2	496.1	248.0	0.42	0.658
Day.Format. Length of shank	5	1392.7	278.5	0.47	0.798
Day.Format.Tissue	10	12006.1	1200.6	2.03	0.032
Day. Length of shank.Tissue	10	2380.0	238.0	0.40	0.945
Format. Length of shank.Tissue	2	2858.4	1429.2	2.41	0.092
Residual	195	115478.9	592.2		
Total	256	479889.7			

**Table B.31** Total sugar concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	66120.	13224.	2.73	0.021
Format	1	4780.	4780.	0.99	0.322
Length of shank	1	6745.	6745.	1.39	0.240
Tissue	2	199277.	99639.	20.54	<.001
Day.Format	5	31379.	6276.	1.29	0.268
Day.Length of shank	5	24774.	4955.	1.02	0.406
Format.Length of shank	1	778.	778.	0.16	0.689
Day.Tissue	10	217691.	21769.	4.49	<.001
Format.Tissue	2	43535.	21768.	4.49	0.012
Length of shank.Tissue	2	7570.	3785.	0.78	0.460

**Table B.31** Total sugar concentration [Chapter 6, Experiment 4 (additional variate: length of shank)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format.Length of shank	5	10030.	2006.	0.41	0.839
Day.Format.Tissue	10	126337.	12634.	2.60	0.005
Day.Length of shank.Tissue	10	49795.	4979.	1.03	0.423
Format.Length of shank.Tissue	2	11803.	5901.	1.22	0.299
Residual	195	946069.	4852.		
Total	256	1639572.			

**Table B.32** Sucrose concentration [Chapter 6, Experiment 4 (additional variate: type of tissue)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	369465.	184733.	87.89	<.001
Tissue	2	191494.	95747.	45.55	<.001
Position in the cob	2	3818.	1909.	0.91	0.407
Day. Tissue	4	18722.	4680.	2.23	0.073
Day. Position in the cob	4	21804.	5451.	2.59	0.042
Tissue. Position in the cob	4	11958.	2990.	1.42	0.233
Day. Tissue. Position in the cob	8	38713.	4839.	2.30	0.027
Residual	87	182860.	2102.		
Total	113	748961.			

**Table B.33** Glucose concentration [Chapter 6, Experiment 4 (additional variate: type of tissue)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	6019.0	3009.5	9.43	<.001
Tissue	2	104943.9	52472.0	164.45	<.001
Position in the cob	2	4812.9	2406.4	7.54	<.001
Day.Tissue	4	9651.1	2412.8	7.56	<.001
Day.Position in the cob	4	4311.1	1077.8	3.38	0.013
Tissue.Position in the cob	4	6351.9	1588.0	4.98	0.001



**Table B.33** Glucose concentration [Chapter 6, Experiment 4 (additional variate: type of tissue)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day. Tissue.Position in the cob	8	10051.6	1256.4	3.94	<.001
Residual	87	27759.6	319.1		
Total	113	148270.1			

**Table B.34** Fructose concentration [Chapter 6, Experiment 4 (additional variate: type of tissue)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	3789.0	1894.5	8.57	<.001
Tissue	2	72193.4	36096.7	163.21	<.001
Position in the cob	2	3825.6	1912.8	8.65	<.001
Day. Tissue	4	6445.6	1611.4	7.29	<.001
Day.Position in the cob	4	3166.2	791.6	3.58	0.009
Tissue. Position in the cob	4	5273.0	1318.2	5.96	<.001
Day. Tissue.Position in the cob	8	8936.4	1117.1	5.05	<.001
Residual	87	19241.4	221.2		
Total	113	104310.4			

**Table B.35** Total sugars concentration [Chapter 6, Experiment 4 (additional variate: type of tissue)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	466441.	233221.	121.55	<.001
Tissue	2	76058.	38029.	19.82	<.001
Position in the cob	2	5242.	2621.	1.37	0.261
Day. Tissue	4	20744.	5186.	2.70	0.036
Day.Position in the cob	4	39362.	9840.	5.13	<.001
Tissue.Position in the cob	4	12132.	3033.	1.58	0.187
Day. Tissue.Position in the cob	8	59908.	7489.	3.90	<.001
Residual	87	166934.	1919.		
Total	113	726938.			

**Table B.36** Shank-Sucrose concentration [Chapter 6, Experiment 4 (additional variate: position in the cob)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	94032.	47016.	25.23	<.001
Position in the cob	2	2294.	1147.	0.62	0.547
Day.Position in the cob	4	2221.	555.	0.30	0.877
Residual	29	54051.	1864.		
Total	37	146595.			

**Table B.37** Kernels-Sucrose concentration [Chapter 6, Experiment 4]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	73947.	14789.	4.62	0.001
Format	1	366.	366.	0.11	0.736
Day.Format	5	20692.	4138.	1.29	0.277
Residual	74	237039.	3203.		
Total	85	322440.			

**Table B.38** Shank-Glucose concentration [Chapter 6, Experiment 4 (additional variate: position in the cob)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	14064.5	7032.3	21.40	<.001
Position in the cob	2	2454.3	1227.1	3.73	0.036
Day.Position in the cob	4	322.7	80.7	0.25	0.910
Residual	29	9529.3	328.6		
Total	37	24633.5			

**Table B.39** Kernels-Glucose concentration [Chapter 6, Experiment 4]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	5265.3	1053.1	5.02	<.001
Format	1	3.6	3.6	0.02	0.896

**Table B.39** Kernels-Glucose concentration [Chapter 6, Experiment 4]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format	5	1662.7	332.5	1.58	0.175
Residual	74	15536.5	210.0		
Total	85	21454.6			

**Table B.40** Shank-Fructose concentration [Chapter 6, Experiment 4 (additional variate: position in the cob)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	7784.9	3892.5	19.49	<.001
Position in the cob	2	1212.9	606.4	3.04	0.063
Day.Position in the cob	4	193.4	48.4	0.24	0.912
Residual	29	5790.9	199.7		
Total	37	13822.3			

**Table B.41** Kernels-Fructose concentration [Chapter 6, Experiment 4]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	2313.2	462.6	2.10	0.074
Format	1	472.4	472.4	2.15	0.147
Day.Format	5	842.8	168.6	0.77	0.577
Residual	74	16279.6	220.0		
Total	85	19441.1			

**Table B.42** Shank-Total sugar concentration [Chapter 6, Experiment 4 (additional variate: position in the cob)]

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	2	217220.	108610.	43.23	<.001
Position in the cob	2	1388.	694.	0.28	0.761
Day.Position in the cob	4	3590.	897.	0.36	0.837
Residual	29	72865.	2513.		
Total	37	279311.			

**Table B.43**      Kernels-Total sugars concentration [Chapter 6, Experiment 4]

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	36370.	7274.	2.04	0.082
Format	1	0.	0.	0.00	0.991
Day.Format	5	37221.	7444.	2.09	0.076
Residual	74 (10)	263276.	3558.		
Total	85 (10)	327708.			

**Table B.44**      Maximum compressive load (Chapter 6, Experiment 4)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	34.482	6.896	2.73	0.018
Format	1	55.234	55.234	21.85	<.001
Position in cob	3	21.953	7.318	2.89	0.034
Length of shank	1	1.158	1.158	0.46	0.499
Day.Format	5	23.900	4.780	1.89	0.093
Day.Position in cob	15	29.448	1.963	0.78	0.705
Format.Position in cob	3	6.058	2.019	0.80	0.494
Day. Length of shank	5	63.797	12.759	5.05	<.001
Format. Length of shank	1	10.712	10.712	4.24	0.040
Position in cob. Length of shank	3	4.167	1.389	0.55	0.649
Day.Format.Position in cob	15	23.628	1.575	0.62	0.858
Day.Format. Length of shank	5	53.997	10.799	4.27	<.001
Day.Position in cob. Length of shank	15	33.365	2.224	0.88	0.587
Format.Position in cob. Length of shank	3	12.833	4.278	1.69	0.167
Residual	1503	3799.186	2.528		
Total	1583	4134.765			

**Table B.45**      Sucrose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv.	1	45274.	45274.	14.30	<.001
Day	5	99030.	19806.	6.26	<.001
Format	1	534.	534.	0.17	0.682

**Table B.45** Sucrose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Tissue	1	42532.	42532.	13.43	<.001
Position in the cob	2	6184.	3092.	0.98	0.378
Cv.Day	5	13689.	2738.	0.86	0.505
Cv.Format	1	14948.	14948.	4.72	0.030
Day.Format	5	33799.	6760.	2.14	0.061
Cv. Tissue	1	21847.	21847.	6.90	0.009
Day. Tissue	5	63359.	12672.	4.00	0.002
Format. Tissue	1	1697.	1697.	0.54	0.465
Cv.Position in the cob	2	7438.	3719.	1.17	0.310
Day.Position in the cob	10	45388.	4539.	1.43	0.164
Format.Position in the cob	2	13247.	6623.	2.09	0.125
part.Position in the cob	2	27254.	13627.	4.30	0.014
Cv.Day.Format	5	28473.	5695.	1.80	0.112
Cv.Day. Tissue	5	35950.	7190.	2.27	0.047
Cv.Format. Tissue	1	9556.	9556.	3.02	0.083
Day.Format. Tissue	5	13067.	2613.	0.83	0.532
Cv.Day.Position in the cob	10	61339.	6134.	1.94	0.039
Cv.Format.Position in the cob	2	6391.	3196.	1.01	0.366
Day.Format.Position in the cob	10	95998.	9600.	3.03	0.001
Cv. Tissue.position	2	6632.	3316.	1.05	0.352
Day. Tissue.Position in the cob	10	47395.	4740.	1.50	0.139
Format. Tissue.Position in the cob	2	1695.	848.	0.27	0.765
Residual	350	1108160.	3166.		
Total	446	1682006.			

**Table B.46** Glucose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv	1	10450.	10450.	4.69	0.031
Day	5	245801.	49160.	22.05	<.001
Format	1	12493.	12493.	5.60	0.018
Tissue	1	152007.	152007.	68.17	<.001
Position in the cob	2	37028.	18514.	8.30	<.001

**Table B.46** Glucose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv.Day	5	92009.	18402.	8.25	<.001
Cv.Format	1	176.	176.	0.08	0.779
Day.Format	5	17528.	3506.	1.57	0.167
Cv. Tissue	1	10533.	10533.	4.72	0.030
Day. Tissue	5	165588.	33118.	14.85	<.001
Format. Tissue	1	3163.	3163.	1.42	0.234
Cv.Position in the cob	2	2789.	1394.	0.63	0.536
Day.Position in the cob	10	45216.	4522.	2.03	0.030
Format.Position in the cob	2	8926.	4463.	2.00	0.137
part.Position in the cob	2	44047.	22024.	9.88	<.001
Cv.Day.Format	5	57344.	11469.	5.14	<.001
Cv.Day. Tissue	5	88675.	17735.	7.95	<.001
Cv.Format. Tissue	1	7371.	7371.	3.31	0.070
Day.Format. Tissue	5	11747.	2349.	1.05	0.386
Cv.Day.Position in the cob	10	34807.	3481.	1.56	0.117
Cv.Format.Position in the cob	2	1711.	855.	0.38	0.682
Day.Format.Position in the cob	10	56546.	5655.	2.54	0.006
Cv. Tissue.Position in the cob	2	13023.	6512.	2.92	0.055
Day. Tissue.Position in the cob	10	26337.	2634.	1.18	0.302
Format. Tissue.Position in the cob	2	7512.	3756.	1.68	0.187
Residual	350	780470.	2230.		
Total	446	1661158.			

**Table B.47** Fructose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv	1	2.6	2.6	0.01	0.933
Day	5	9513.1	1902.6	5.16	<.001
Format	1	3790.6	3790.6	10.28	0.001
Tissue	1	221596.6	221596.6	601.14	<.001
Position in the cob	2	29574.5	14787.3	40.11	<.001
Cv.Day	5	1197.8	239.6	0.65	0.662

**Table B.47** Fructose concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv.Format	1	3426.9	3426.9	9.30	0.002
Day.Format	5	256.9	51.4	0.14	0.983
Cv. Tissue	1	708.5	708.5	1.92	0.167
Day. Tissue	5	10207.9	2041.6	5.54	<.001
Format. Tissue	1	1125.7	1125.7	3.05	0.081
Cv.Position in the cob	2	1273.9	636.9	1.73	0.179
Day.Position in the cob	10	11013.7	1101.4	2.99	0.001
Format.Position in the cob	2	612.9	306.5	0.83	0.436
part.Position in the cob	2	11092.7	5546.3	15.05	<.001
Cv.Day.Format	5	2039.3	407.9	1.11	0.357
Cv.Day. Tissue	5	3401.4	680.3	1.85	0.103
Cv.Format. Tissue	1	4025.7	4025.7	10.92	0.001
Day.Format. Tissue	5	3150.1	630.0	1.71	0.132
Cv.Day.Position in the cob	10	3730.6	373.1	1.01	0.433
Cv.Format.Position in the cob	2	2817.1	1408.5	3.82	0.023
Day.Format.Position in the cob	10	7376.6	737.7	2.00	0.032
Cv. Tissue. Position in the cob	2	2125.4	1062.7	2.88	0.057
Day. Tissue.Position in the cob	10	8908.0	890.8	2.42	0.009
Format. Tissue.Position in the cob	2	3835.2	1917.6	5.20	0.006
Residual	350 (129)	129019.8	368.6		
Total	446 (129)	402431.2			

**Table B.48** Total sugars concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cv.	1	12579.	12579.	2.05	0.153
Day	5	319589.	63918.	10.41	<.001
Format	1	38591.	38591.	6.29	0.013
Tissue	1	1138180.	1138180.	185.41	<.001
Position in the cob	2	85182.	42591.	6.94	0.001
Cv.Day	5	69065.	13813.	2.25	0.049
Cv.Format	1	28068.	28068.	4.57	0.033

**Table B.48** Total sugars concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format	5	68297.	13659.	2.23	0.051
Cv. Tissue	1	50095.	50095.	8.16	0.005
Day. Tissue	5	126554.	25311.	4.12	0.001
Format. Tissue	1	2361.	2361.	0.38	0.536
Cv.Position in the cob	2	1575.	787.	0.13	0.880
Day.Position in the cob	10	137492.	13749.	2.24	0.015
Format.Position in the cob	2	6122.	3061.	0.50	0.608
part.Position in the cob	2	164190.	82095.	13.37	<.001
Cv.Day.Format	5	125807.	25161.	4.10	0.001
Cv.Day. Tissue	5	155878.	31176.	5.08	<.001
Cv.Format. Tissue	1	2657.	2657.	0.43	0.511
Day.Format. Tissue	5	32922.	6584.	1.07	0.375
Cv.Day.Position in the cob	10	89291.	8929.	1.45	0.155
Cv.Format.Position in the cob	2	6879.	3440.	0.56	0.572
Day.Format.Position in the cob	10	171086.	17109.	2.79	0.002
Cv. Tissue.Position in the cob	2	48093.	24046.	3.92	0.021
Day. Tissue.Position in the cob	10	110608.	11061.	1.80	0.059
Format.Tissue.Position in the cob	2	6120.	3060.	0.50	0.608
Residual	350	2148536.	6139.		
Total	446	4440684.			

**Table B.49** Weight loss % (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	1	0.000	0.000	0.00	0.999
Days	5	17.792	3.558	1.82	0.121
Format	1	7.366	7.366	3.76	0.057
Cultivars.Days	5	13.166	2.633	1.34	0.257
Cultivars.Format	1	0.005	0.005	0.00	0.960
Days.Format	5	13.852	2.770	1.41	0.230
Cultivars.Days.Format	5	12.576	2.515	1.28	0.281
Residual	67	131.258	1.959		
Total	90	186.162			



**Table B.50** Maximum compressive load (Chapter 6, Experiment 5)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cv.	1		0.050	0.050	0.02	0.875
Day	5		144.086	28.817	14.14	<.001
Format	1		34.458	34.458	16.91	<.001
Position in cob	2		478.147	239.074	117.33	<.001
Cv.Day	5		52.295	10.459	5.13	<.001
Cv.Format	1		6.159	6.159	3.02	0.082
Day.Format	5		11.463	2.293	1.13	0.345
Cv.Position in cob	2		13.013	6.506	3.19	0.041
Day. Position in cob	10		63.111	6.311	3.10	<.001
Format. Position in cob	2		1.410	0.705	0.35	0.708
Cv.Day.Format	5		24.580	4.916	2.41	0.035
Cv.Day. Position in cob	10		19.540	1.954	0.96	0.478
Cv.Format.Position in cob	2		12.881	6.440	3.16	0.043
Day.Format.Position in cob	10		54.691	5.469	2.68	0.003
Residual	1004	(86)	2045.827	2.038		
Total	1065	(86)	2898.777			

**Table B.51** CO<sub>2</sub> concentration (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	1	74.920	74.920	8.16	0.006
Days	5	452.930	90.586	9.87	<.001
Format	1	5.768	5.768	0.63	0.431
Cultivars.Days	5	29.733	5.947	0.65	0.664
Cultivars.Format	1	43.082	43.082	4.70	0.035
Days.Format	5	66.742	13.348	1.45	0.219
Cultivars.Days.Format	5	53.930	10.786	1.18	0.333
Residual	56	513.863	9.176		
Total	79	1106.399			

**Table B.52** Moisture content (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivar	1	400.79	400.79	30.83	<.001
Day	4	48.85	12.21	0.94	0.442
Format	1	8.76	8.76	0.67	0.413
Part	1	8722.84	8722.84	670.91	<.001
Position in cob	2	753.32	376.66	28.97	<.001
Cultivar.Day	4	24.39	6.10	0.47	0.759
Cultivar.Format	1	0.18	0.18	0.01	0.906
Day.Format	4	37.16	9.29	0.71	0.583
Cultivar.Part	1	122.79	122.79	9.44	0.002
Day.Part	4	88.02	22.01	1.69	0.152
Format.Part	1	37.98	37.98	2.92	0.089
Cultivar.Position in cob	2	3.91	1.95	0.15	0.861
Day.Position in cob	8	155.61	19.45	1.50	0.159
Format.Position in cob	2	31.86	15.93	1.23	0.295
Part.Position in cob	2	95.89	47.95	3.69	0.026
Cultivar.Day.Format	4	132.27	33.07	2.54	0.040
Cultivar.Day.Part	4	74.02	18.51	1.42	0.227
Cultivar.Format.Part	1	48.73	48.73	3.75	0.054
Day.Format.Part	4	35.39	8.85	0.68	0.606
Cultivar.Day.Position in cob	8	116.20	14.52	1.12	0.352
Cultivar.Format.Position in cob	2	3.99	1.99	0.15	0.858
Day.Format.Position in cob	8	89.50	11.19	0.86	0.551
Cultivar.Part.Position in cob	2	13.89	6.94	0.53	0.587
Day.Part.Position in cob	8	256.47	32.06	2.47	0.014
Format.Part.Position in cob	2	19.77	9.88	0.76	0.469
Residual	251	3263.38	13.00		
Total	332	9880.67			

**Table B.53** Moisture content of core (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	31.45	7.86	0.29	0.882

**Table B.53** Moisture content of core (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Format	1	164.78	164.78	6.14	0.014
Cultivar	1	107.95	107.95	4.02	0.047
Day.Format	4	153.05	38.26	1.43	0.229
Day. Cultivar	4	55.44	13.86	0.52	0.724
Format. Cultivar	1	106.79	106.79	3.98	0.048
Day.Format. Cultivar	4	117.94	29.49	1.10	0.360
Residual	139	3732.11	26.85		
Total	158	4194.21			

**Table B.54** Moisture content of kernels (Chapter 6, Experiment 5)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	18.436	4.609	0.54	0.704
Format	1	2.883	2.883	0.34	0.560
Var	1	595.622	595.622	70.31	<.001
Day.Format	4	27.556	6.889	0.81	0.519
Day.Var	4	48.634	12.158	1.44	0.225
Format.Var	1	54.147	54.147	6.39	0.012
Day.Format.Var	4	38.297	9.574	1.13	0.344
Residual	154	1304.556	8.471		
Total	173	1824.711			

**Table B.55** Hue angle (Chapter 7)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		142.05	28.41	1.31	0.267
Residual	112	(2)	2436.63	21.76		
Total	117	(2)	2578.16			

**Table B.56** Lightness (Chapter 7)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		361.341	72.268	12.47	<.001
Residual	112	(2)	648.927	5.794		
Total	117	(2)	1009.163			

**Table B.57** Chroma (Chapter 7)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5		607.448	121.490	28.19	<.001
Residual	112	(2)	482.652	4.309		
Total	117	(2)	1089.657			

**Table B.58** CO<sub>2</sub> (%) (Chapter 6)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	67.701	13.540	5.11	0.004
Residual	18	47.659	2.648		
Total	23	115.360			

**Table B.59** Maximum compressive load (Chapter 7)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Cooking time	4		1862.339	465.585	239.69	<.001
Day	5		63.603	12.721	6.55	<.001
Cooking time.Day	20		67.548	3.377	1.74	0.023
Residual	1041	(9)	2022.109	1.942		
Total	1070	(9)	4015.152			

**Table B.60** Weight loss (Chapter 7)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4		9.968	2.492	0.93	0.451
Cooking time	4		10.937	2.734	1.02	0.403

**Table B.60** Weight loss (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day. Cooking time	16	42.702	2.669	1.00	0.471
Residual	74 (1)	198.315	2.680		
Total	98 (1)	261.869			

**Table B.61** Moisture content (Chapter 7)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	58.368	11.674	8.32	<.001
Cooking time	4	336.158	84.040	59.89	<.001
Day.Cooking time	20	35.389	1.769	1.26	0.227
Residual	90	126.286	1.403		
Total	119	556.202			

**Table B.62** Starch (Chapter 7)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	376.928	75.386	10.81	<.001
Cooking time	4	408.165	102.041	14.63	<.001
Day. Cooking time	20	132.772	6.639	0.95	0.529
Residual	60	418.351	6.973		
Total	89	1336.216			

**Table B.63** Sucrose (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	40527.9	8105.6	15.11	<.001
Cooking time	4	253.0	63.2	0.12	0.976
Day.Cooking_time	20	14356.0	717.8	1.34	0.180
Residual	83 (7)	44538.8	536.6		
Total	112 (7)	98660.9			

**Table B.64** Glucose (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	2679.79	535.96	22.52	<.001
Cooking_time	4	173.71	43.43	1.82	0.132
Day.Cooking_time	20	1692.85	84.64	3.56	<.001
Residual	83 (7)	1975.29	23.80		
Total	112 (7)	6322.51			

**Table B.65** Fructose (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	3385.852	677.170	360.60	<.001
Cooking time	4	40.625	10.156	5.41	<.001
Day.Cooking time	20	912.273	45.614	24.29	<.001
Residual	83 (7)	155.866	1.878		
Total	112 (7)	4294.718			

**Table B.66** Total sugars (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	80548.5	16109.7	24.82	<.001
Cooking_time	4	670.2	167.6	0.26	0.904
Day.Cooking time	20	27543.9	1377.2	2.12	0.009
Residual	83 (7)	53881.9	649.2		
Total	112 (7)	159930.4			

**Table B.67** Sweetness (Chapter 7)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	5	79083.5	15816.7	25.56	<.001
Cooking time	4	464.8	116.2	0.19	0.944
Day.Cooking time	20	25636.3	1281.8	2.07	0.012
Residual	83 (7)	51369.6	618.9		
Total	112 (7)	153903.8			

**Table B.68** Total phenolics on dry weight basis (Chapter 7)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	17.7789	3.5558	27.54	<.001
Cooking time	4	3.1765	0.7941	6.15	<.001
Day. Cooking time	20	14.6459	0.7323	5.67	<.001
Residual	60	7.7481	0.1291		
Total	89	43.3494			

**Table B.69** Total phenolics on fresh weight basis (Chapter 7)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	5	1.700847	0.340169	38.19	<.001
Cooking time	4	0.758167	0.189542	21.28	<.001
Day. Cooking time	20	1.312118	0.065606	7.37	<.001
Residual	60	0.534429	0.008907		
Total	89	4.305562			

**Table B.70** Hue angle of kernels (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	18.8168	4.7042	5.46	<.001
Format	1	143.1134	143.1134	166.14	<.001
Temperature	1	0.0083	0.0083	0.01	0.922
Cooking_time	2	65.7656	32.8828	38.17	<.001
Day.Format	4	2.8444	0.7111	0.83	0.510
Day.Temperature	4	1.9024	0.4756	0.55	0.698
Format.Temperature	1	0.0019	0.0019	0.00	0.963
Day. Cooking_time	8	15.2507	1.9063	2.21	0.028
Format. Cooking_time	2	3.4057	1.7028	1.98	0.141
Temp. Cooking_time	2	2.1140	1.0570	1.23	0.295
Day.Format. Temperature	4	1.4805	0.3701	0.43	0.787
Day.Format. Cooking_time	8	13.0263	1.6283	1.89	0.064
Day. Temperature. Cooking_time	8	6.3579	0.7947	0.92	0.499
Format. Temperature. Cooking_time	2	0.9237	0.4618	0.54	0.586

**Table B.70** Hue angle of kernels (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Residual	188	161.9424	0.8614		
Total	239	436.9539			

**Table B.71** Hue angle of husks (Chapter 8)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4		78.735	19.684	3.00	0.034
Temperature	1		80.798	80.798	12.33	0.001
Day. Temperature	4		16.859	4.215	0.64	0.636
Residual	29	(1)	190.073	6.554		
Total	38	(1)	359.755			

**Table B.72** Lightness of kernels (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	520.857	130.214	29.03	<.001
Format	1	8.125	8.125	1.81	0.180
Temperature	1	1.140	1.140	0.25	0.615
Cooking time	2	1043.772	521.886	116.36	<.001
Day.Format	4	33.427	8.357	1.86	0.119
Day. Temperature	4	23.294	5.823	1.30	0.272
Format. Temperature	1	18.805	18.805	4.19	0.042
Day. Cooking time	8	134.651	16.831	3.75	<.001
Format. Cooking time	2	1.784	0.892	0.20	0.820
Temperature. Cooking time	2	10.797	5.399	1.20	0.302
Day.Format. Temperature	4	26.703	6.676	1.49	0.207
Day.Format. Cooking time	8	63.723	7.965	1.78	0.084
Day. Temperature. Cooking time	8	56.660	7.083	1.58	0.133
Format. Temperature. Cooking time	2	15.375	7.688	1.71	0.183
Residual	188	843.197	4.485		
Total	239	2802.311			



**Table B.73** Lightness of husks (Chapter 8)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	320.23	80.06	2.56	0.060
Temperature	1	0.69	0.69	0.02	0.883
Day. Temperature	4	111.95	27.99	0.89	0.481
Residual	29 (1)	908.55	31.33		
Total	38 (1)	1311.12			

**Table B.74** Chroma of kernels (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	198.896	49.724	6.82	<.001
Format	1	36.069	36.069	4.94	0.027
Temperature	1	42.135	42.135	5.78	0.017
Cooking time	2	12735.054	6367.527	872.85	<.001
Day.Format	4	50.202	12.551	1.72	0.147
Day. Temperature	4	52.085	13.021	1.78	0.134
Format. Temperature	1	47.669	47.669	6.53	0.011
Day. Cooking time	8	84.696	10.587	1.45	0.178
Format. Cooking time	2	80.090	40.045	5.49	0.005
Temperature. Cooking time	2	62.214	31.107	4.26	0.015
Day.Format. Temperature	4	27.398	6.849	0.94	0.443
Day.Format. Cooking time	8	133.581	16.698	2.29	0.023
Day. Temperature. Cooking time	8	72.172	9.021	1.24	0.280
Format. Temperature. Cooking time	2	6.719	3.360	0.46	0.632
Residual	188	1371.476	7.295		
Total	239	15000.456			

**Table B.75** Chroma of husks (Chapter 8)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4	47.548	11.887	1.99	0.122
Temp	1	31.607	31.607	5.30	0.029
Day. Temperature	4	8.764	2.191	0.37	0.830

**Table B.75** Chroma of husks (Chapter 8)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Residual	29	(1)	172.906	5.962		
Total	38	(1)	259.448			

**Table B.76** Ferulic acid (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	8.12272	2.03068	23.54	<.001
Format	1	2.39000	2.39000	27.71	<.001
Temperature	1	0.42427	0.42427	4.92	0.028
Cooking_time	2	0.66615	0.33308	3.86	0.024
Day.Format	4	0.21233	0.05308	0.62	0.652
Day. Temperature	4	0.25997	0.06499	0.75	0.557
Format. Temperature	1	0.35604	0.35604	4.13	0.044
Day. Cooking time	8	0.88888	0.11111	1.29	0.255
Format. Cooking_time	2	0.01360	0.00680	0.08	0.924
Temperature. Cooking_time	2	0.05739	0.02869	0.33	0.718
Day.Format. Temperature	4	1.13479	0.28370	3.29	0.013
Day.Format. Cooking_time	8	0.33600	0.04200	0.49	0.864
Day. Temperature. Cooking_time	8	0.94752	0.11844	1.37	0.214
Format. Temperature. Cooking_time	2	0.57820	0.28910	3.35	0.038
Residual	128	11.04083	0.08626		
Total	179	27.42867			

**Table B.77** Frap (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cooking time	2	21.352	10.676	1.26	0.286
Day	4	50.919	12.730	1.51	0.204
Format	1	109.998	109.998	13.02	<.001
Temperature	1	8.437	8.437	1.00	0.320
Cooking time. Day	8	105.384	13.173	1.56	0.144
Cooking time.Format	2	2.311	1.156	0.14	0.872

**Table B.77** Frap (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format	4	38.913	9.728	1.15	0.336
Cooking time.temp	2	65.139	32.569	3.85	0.024
Day. Temperature	4	6.098	1.525	0.18	0.948
Format. Temperature	1	15.548	15.548	1.84	0.177
Cooking time.Day.Format	8	55.197	6.900	0.82	0.589
Cooking time.Day. Temperature	8	130.907	16.363	1.94	0.060
Cooking time.Format. Temperature	2	0.075	0.037	0.00	0.996
Day.format. Temperature	4	35.480	8.870	1.05	0.384
Residual	128	1081.661	8.450		
Total	179	1727.420			

**Table B.78** Total soluble solids (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	26.885	6.721	6.38	<.001
Format	1	1.582	1.582	1.50	0.223
Temperature	1	0.373	0.373	0.35	0.553
Cooking time	1	0.063	0.063	0.06	0.807
Day.Format	4	9.711	2.428	2.30	0.062
Day. Temperature	4	6.426	1.606	1.53	0.199
Format. Temperature	1	0.063	0.063	0.06	0.807
Day. Cooking time	4	28.378	7.095	6.74	<.001
Format. Cooking time	1	1.582	1.582	1.50	0.223
Temperature. Cooking time	1	0.569	0.569	0.54	0.464
Day.Format. Temperature	4	4.850	1.213	1.15	0.336
Day.Format. Cooking time	4	3.848	0.962	0.91	0.458
Day. Temperature. Cooking time	4	4.344	1.086	1.03	0.394
Format. Temperature. Cooking time	1	8.059	8.059	7.65	0.007
Residual	124	130.610	1.053		
Total	159	227.344			

**Table B.79** Volume remained in the microwable bowl (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	12.713	3.178	0.89	0.474
Format	1	3.025	3.025	0.84	0.360
Temperature	1	5.625	5.625	1.57	0.213
Cooking time	1	1476.225	1476.225	411.66	<.001
Day.Format	4	46.037	11.509	3.21	0.015
Day. Temperature	4	82.938	20.734	5.78	<.001
Format. Temperature	1	42.025	42.025	11.72	<.001
Day. Cooking time	4	18.213	4.553	1.27	0.286
Format. Cooking time	1	4.225	4.225	1.18	0.280
Temp. Cooking time	1	0.225	0.225	0.06	0.803
Day.Format. Temperature	4	40.663	10.166	2.83	0.027
Day.Format. Cooking time	4	71.837	17.959	5.01	<.001
Day.Temp. Cooking time	4	16.338	4.084	1.14	0.341
Format. Temperature. Cooking time	1	11.025	11.025	3.07	0.082
Residual	124	444.662	3.586		
Total	159	2275.775			

**Table B.80** Sucrose (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	56428.1	14107.0	20.41	<.001
Format	1	78084.5	78084.5	112.96	<.001
Temperature	1	789.1	789.1	1.14	0.287
Cooking time	2	2163.8	1081.9	1.57	0.213
Day.Format	4	8466.6	2116.7	3.06	0.019
Day. Temperature	4	2397.5	599.4	0.87	0.486
Format. Temperature	1	4529.6	4529.6	6.55	0.012
Day. Cooking time	8	9173.6	1146.7	1.66	0.115
Format. Cooking time	2	2373.9	1186.9	1.72	0.184
Temperature. Cooking time	2	865.9	432.9	0.63	0.536
Day.Format. Temperature	4	5176.5	1294.1	1.87	0.119

**Table B.80** Sucrose (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format. Cooking time	8	9737.6	1217.2	1.76	0.091
Day. Temperature. Cooking time	8	3227.1	403.4	0.58	0.790
Format. Temperature. Cooking time	2	164.4	82.2	0.12	0.888
Residual	128	88478.7	691.2		
Total	179	272056.9			

**Table B.81** Glucose (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	751.97	187.99	7.55	<.001
Format	1	2662.50	2662.50	107.00	<.001
Temperature	1	50.61	50.61	2.03	0.156
Cooking time	2	656.94	328.47	13.20	<.001
Day.Format	4	156.52	39.13	1.57	0.186
Day. Temperature	4	427.29	106.82	4.29	0.003
Format. Temperature	1	181.69	181.69	7.30	0.008
Day. Cooking time	8	466.26	58.28	2.34	0.022
Format. Cooking time	2	23.32	11.66	0.47	0.627
Temperature. Cooking time	2	4.77	2.39	0.10	0.909
Day.Format. Temperature	4	194.42	48.60	1.95	0.106
Day.Format. Cooking time	8	191.97	24.00	0.96	0.467
Day. Temperature. Cooking time	8	187.08	23.38	0.94	0.486
Format. Temperature. Cooking time	2	6.20	3.10	0.12	0.883
Residual	128	3185.08	24.88		
Total	179	9146.63			

**Table B.82** Fructose (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	458.150	114.537	13.47	<.001
Format	1	285.487	285.487	33.57	<.001

**Table B.82** Fructose (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Temperature	1	17.166	17.166	2.02	0.158
Cooking time	2	266.756	133.378	15.68	<.001
Day.Format	4	110.431	27.608	3.25	0.014
Day. Temperature	4	382.492	95.623	11.24	<.001
Format. Temperature	1	74.136	74.136	8.72	0.004
Day. Cooking time	8	294.855	36.857	4.33	<.001
Format. Cooking time	2	13.064	6.532	0.77	0.466
Temperature. Cooking time	2	10.701	5.350	0.63	0.535
Day.Format. Temperature	4	99.597	24.899	2.93	0.023
Day.Format. Cooking time	8	40.789	5.099	0.60	0.777
Day. Temperature. Cooking time	8	61.450	7.681	0.90	0.516
Format. Temperature. Cooking time	2	11.523	5.761	0.68	0.510
Residual	128	1088.550	8.504		
Total	179	3215.146			

**Table B.83** Total sugars (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	71317.6	17829.4	19.61	<.001
Format	1	121056.6	121056.6	133.18	<.001
Temperature	1	1548.3	1548.3	1.70	0.194
Cooking time	2	2353.8	1176.9	1.29	0.278
Day.Format	4	11276.0	2819.0	3.10	0.018
Day. Temperature	4	2569.0	642.3	0.71	0.589
Format.Temp	1	7990.9	7990.9	8.79	0.004
Day. Cooking time	8	10491.6	1311.5	1.44	0.185
Format. Cooking time	2	1772.0	886.0	0.97	0.380
Temp. Cooking time	2	821.1	410.5	0.45	0.638
Day.Format. Temperature	4	5844.4	1461.1	1.61	0.176
Day.Format. Cooking time	8	12686.9	1585.9	1.74	0.094
Day. Temperature. Cooking time	8	4488.2	561.0	0.62	0.762
Format. Temperature. Cooking time	2	66.0	33.0	0.04	0.964

**Table B.83** Total sugars (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Residual	128	116350.2	909.0		
Total	179	370632.7			

**Table B.84** Maximum compressive load (Chapter 8)

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
Day	4		11.7669	2.9417	3.19	0.013
Format	1		29.2549	29.2549	31.73	<.001
Cooking time	2		1209.6743	604.8371	656.10	<.001
Day.Format	4		8.6637	2.1659	2.35	0.052
Day. Temperature	4		16.5783	4.1446	4.50	0.001
Format. Temperature	1		4.2421	4.2421	4.60	0.032
Day. Cooking time	8		27.0889	3.3861	3.67	<.001
Format. Cooking time	2		0.5110	0.2555	0.28	0.758
Temperature. Cooking time	2		3.5815	1.7907	1.94	0.144
Day.Format. Temperature	4		3.8834	0.9708	1.05	0.378
Day.Format. Cooking time	8		11.0733	1.3842	1.50	0.152
Day. Temperature. Cooking time	8		12.6651	1.5831	1.72	0.090
Format. Temperature. Cooking time	2		7.1278	3.5639	3.87	0.021
Residual	1292	(97)	1191.0443	0.9219		
Total	1342	(97)	2493.3409			

**Table B.85** Weight loss (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	71.7284	17.9321	46.49	<.001
Format	1	4.3081	4.3081	11.17	<.001
Temperature	1	19.6453	19.6453	50.93	<.001
Day.Format	4	1.6727	0.4182	1.08	0.365
Day.Temperature	4	13.6541	3.4135	8.85	<.001
Format.Temperature	1	0.1120	0.1120	0.29	0.591

**Table B.85** Weight loss (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format.Temperature	4	3.2955	0.8239	2.14	0.077
Residual	220	84.8630	0.3857		
Total	239	199.2791			

**Table B.86** L-Ascorbic acid FW (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	34.655	8.664	6.65	<.001
Format	1	0.749	0.749	0.57	0.450
Temperature	1	0.611	0.611	0.47	0.495
cook_time	2	13.999	6.999	5.37	0.006
Day.Format	4	6.522	1.630	1.25	0.293
Day. Temperature	4	2.748	0.687	0.53	0.716
Format. Temperature	1	13.211	13.211	10.14	0.002
Day. Cooking time	8	4.100	0.513	0.39	0.923
Format. Cooking time	2	1.751	0.876	0.67	0.512
Temp. Cooking time	2	0.920	0.460	0.35	0.703
Day.Format. Temperature	4	7.063	1.766	1.35	0.253
Day.Format.cook_time	8	9.930	1.241	0.95	0.476
Day. Temperature. Cooking time	8	6.990	0.874	0.67	0.717
Format. Temperature. Cooking time	2	0.931	0.465	0.36	0.700
Residual	128	166.808	1.303		
Total	179	270.989			

**Table B.87** L-Ascorbic acid DW (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	565.64	141.41	6.95	<.001
Format	1	0.68	0.68	0.03	0.855
Temperature	1	3.92	3.92	0.19	0.661
Cooking time	2	533.27	266.64	13.11	<.001
Day.Format	4	80.43	20.11	0.99	0.416



**Table B.87** L-Ascorbic acid DW (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day. Temperature	4	69.74	17.43	0.86	0.492
Format. Temperature	1	190.60	190.60	9.37	0.003
Day. Cooking time	8	74.36	9.30	0.46	0.884
Format. Cooking time	2	32.66	16.33	0.80	0.450
Temperature. Cooking time	2	25.01	12.50	0.61	0.542
Day.Format. Temperature	4	130.12	32.53	1.60	0.178
Day.Format. Cooking time	8	152.67	19.08	0.94	0.487
Day. Temperature. Cooking time	8	98.82	12.35	0.61	0.770
Format. Temperature. Cooking time	2	14.28	7.14	0.35	0.705
Residual	128	2602.71	20.33		
Total	179	4574.91			

**Table C.88** Total phenolics (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	6.20969	1.55242	21.82	<.001
Format	1	0.31584	0.31584	4.44	0.037
Temperature	1	0.01410	0.01410	0.20	0.657
cook_time	2	0.98970	0.49485	6.96	0.001
Day.Format	4	0.28760	0.07190	1.01	0.404
Day. Temperature	4	0.30586	0.07647	1.07	0.372
Format. Temperature	1	0.00024	0.00024	0.00	0.954
Day. Cooking time	8	2.44745	0.30593	4.30	<.001
Format. Cooking time	2	0.09122	0.04561	0.64	0.528
Temp. Cooking time	2	0.46192	0.23096	3.25	0.042
Day.Format. Temperature	4	0.26679	0.06670	0.94	0.444
Day.Format. Cooking time	8	0.30361	0.03795	0.53	0.829
Day. Temperature. Cooking time	8	1.94276	0.24284	3.41	0.001
Format. Temperature. Cooking time	2	0.47386	0.23693	3.33	0.039
Residual	128	9.10473	0.07113		
Total	179	23.21536			

**Table B.89** Moisture content (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	17.749	4.437	1.49	0.209
Format	1	76.364	76.364	25.65	<.001
Temperature	1	11.403	11.403	3.83	0.053
cooking time	2	372.185	186.092	62.51	<.001
Day.Format	4	18.020	4.505	1.51	0.202
Day. Temperature	4	21.969	5.492	1.84	0.124
Format. Temperature	1	0.790	0.790	0.27	0.607
Day.cooking time	8	55.837	6.980	2.34	0.022
Format. cooking time	2	3.403	1.701	0.57	0.566
Temp. cooking time	2	11.712	5.856	1.97	0.144
Day.Format. Temperature	4	2.215	0.554	0.19	0.945
Day.Format. cooking time	8	20.296	2.537	0.85	0.559
Day. Temperature. cooking time	8	15.357	1.920	0.64	0.739
Format.Temperature. cooking time	2	0.042	0.021	0.01	0.993
Residual	128	381.073	2.977		
Total	179	1008.413			

**Table B.90** Lutein (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	303.84	75.96	6.36	<.001
Format	1	477.55	477.55	39.99	<.001
Temperature	1	157.31	157.31	13.17	<.001
Cooking time	2	810.60	405.30	33.94	<.001
Day.Format	4	21.66	5.42	0.45	0.770
Day. Temperature	4	40.78	10.19	0.85	0.494
Format. Temperature	1	0.12	0.12	0.01	0.922
Day. Cooking time	8	258.78	32.35	2.71	0.009
Format. Cooking time	2	29.94	14.97	1.25	0.289
Temperature. Cooking time	2	48.94	24.47	2.05	0.133

**Table B.90** Lutein (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day.Format. Temperature	4	183.85	45.96	3.85	0.005
Day.Format. Cooking time	8	99.94	12.49	1.05	0.405
Day. Temperature. Cooking time	8	186.93	23.37	1.96	0.057
Format. Temperature. Cooking time	2	22.49	11.25	0.94	0.393
Residual	128	1528.53	11.94		
Total	179	4171.25			

**Table B.91** Zeaxanthin (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	99.543	24.886	21.42	<.001
Format	1	49.176	49.176	42.32	<.001
Temperature	1	0.003	0.003	0.00	0.961
Cooking time	2	19.796	9.898	8.52	<.001
Day.Format	4	28.637	7.159	6.16	<.001
Day. Temperature	4	36.225	9.056	7.79	<.001
Format. Temperature	1	1.654	1.654	1.42	0.235
Day. Cooking time	8	20.876	2.610	2.25	0.028
Format. Cooking time	2	2.386	1.193	1.03	0.361
Temp. Cooking time	2	10.268	5.134	4.42	0.014
Day.Format. Temperature	4	6.175	1.544	1.33	0.263
Day.Format. Cooking time	8	12.273	1.534	1.32	0.239
Day. Temperature. Cooking time	8	9.573	1.197	1.03	0.417
Format. Temperature. Cooking time	2	3.659	1.829	1.57	0.211
Residual	128	148.736	1.162		
Total	179	448.979			

**Table B.92** Total xanthophylls (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	219.27	54.82	4.12	0.004

**Table B.92** Total xanthophylls (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Format	1	833.22	833.22	62.60	<.001
Temperature	1	158.65	158.65	11.92	<.001
Cooking time	2	1082.14	541.07	40.65	<.001
Day.Format	4	78.04	19.51	1.47	0.216
Day. Temperature	4	97.19	24.30	1.83	0.128
Format. Temperature	1	2.64	2.64	0.20	0.657
Day. Cooking time	8	353.63	44.20	3.32	0.002
Format. Cooking time	2	46.24	23.12	1.74	0.180
Temperature. Cooking time	2	89.37	44.68	3.36	0.038
Day.Format. Temperature	4	162.10	40.53	3.04	0.020
Day.Format.cook_time	8	85.24	10.66	0.80	0.603
Day. Temperature. Cooking time	8	233.70	29.21	2.19	0.032
Format.Temperature.Cooking time	2	11.17	5.58	0.42	0.658
Residual	128	1703.61	13.31		
Total	179	5156.23			

**Table B.93** CO<sub>2</sub> (Chapter 8)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Day	4	458.753	114.688	11.90	<.001
Format	1	165.065	165.065	17.13	<.001
Temperature	1	457.197	457.197	47.44	<.001
Day.Format	4	57.556	14.389	1.49	0.216
Day. Temperature	4	147.245	36.811	3.82	0.008
Format. Temperature	1	13.392	13.392	1.39	0.243
Day.Format. Temperature	4	58.645	14.661	1.52	0.207
Residual	60	578.217	9.637		
Total	79	1936.072			

**Table B.94** Sucrose DW (Comparison ELSD-RID methods)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Method	1	14688	14688	3.94	0.082
Cultivar	1	13377	13377	3.59	0.095
Method.Cultivar	1	2667	2667	0.72	0.422
Residual	8	29804	3725		
Total	11	60536			

**Table B.95** Glucose DW (Comparison ELSD-RID methods)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Method	1	95.12	95.12	1.54	0.249
Cultivar	1	682.79	682.79	11.08	0.010
Method.Cultivar	1	102.55	102.55	1.66	0.233
Residual	8	492.93	61.62		
Total	11	1373.38			

**Table B.96** Fructose DW (Comparison ELSD-RID methods)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Method	1	2.23	2.23	0.06	0.817
Cultivar	1	485.57	485.57	12.41	0.008
Method.Cultivar	1	110.53	110.53	2.82	0.131
Residual	8	313.09	39.14		
Total	11	911.42			

**Table B.97** Total sugars DW (Comparison ELSD-RID methods)

Source of variation	d.f. (m.v.)	s.s.	m.s.	v.r.	F pr.
Method	1	17541	17541	3.26	0.109
Cultivar	1	26838	26838	4.99	0.056
Method.Cultivar	1	5225	5225	0.97	0.353
Residual	8	43039	5380		
Total	11	92643			

**Table B.98** L-ascorbic acid (Comparison of FW vs. DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
method	1	2.700987	2.700987	295.65	<.001
Residual	4	0.036544	0.009136		
Total	5	2.737531			

**Table B.99** L-ascorbic acid (Comparison of 20 mg vs. 25 mg vs. 30 mg)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
method	2	0.018281	0.009141	1.18	0.370
Residual	6	0.046521	0.007753		
Total	8	0.064802			

**Table B.100** L-ascorbic acid (Comparison of 25 mg vs. 50 mg vs. 75 mg)

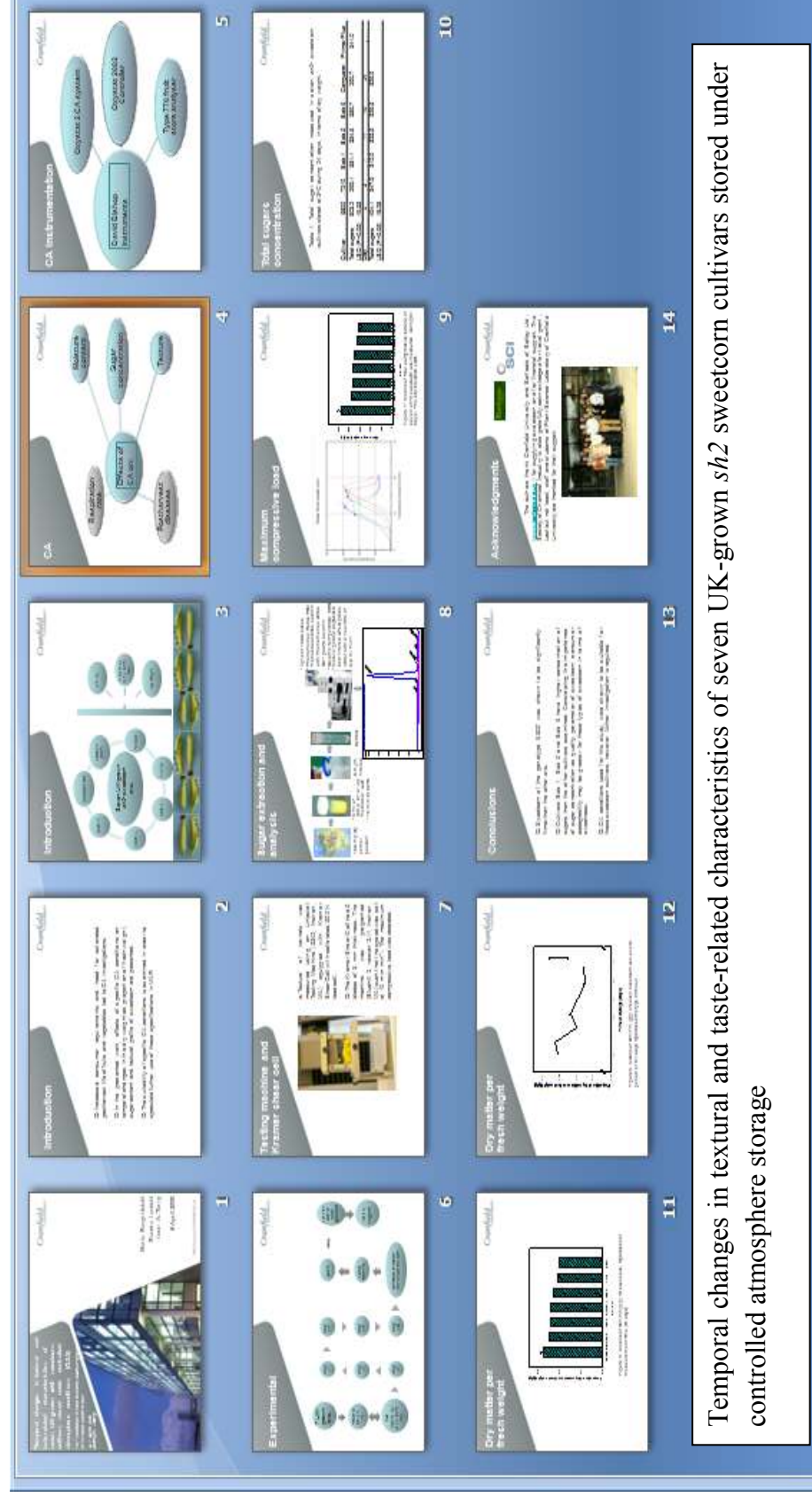
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
method	2	0.049748	0.024874	5.43	0.045
Residual	6	0.027462	0.004577		
Total	8	0.077209			

**Table B.101** L-ascorbic acid (Comparison of 100 mM vs. 1mM of potassium phosphate buffer)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
method	1	0.000656	0.000656	0.28	0.625
Residual	4	0.009365	0.002341		
Total	5	0.010020			

## **Appendix C.**

### **Publications**





**Temporal changes in textural and taste-related characteristics of seven UK-grown *sh2* sweetcorn cultivars stored under controlled atmosphere conditions**

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**Abstract**

Sweetcorn is an important crop in the fresh vegetable market. As consumption increases, greater knowledge is required on quality parameters (*viz.* texture, concentration of sugars) which are intimately associated to consumer acceptability. Deterioration of the quality of sweetcorn after harvest is rapid even for *sh2*-types (super sweet) which tend to deaden the conversion of sugars to starch. Storage at low temperatures after harvest and/or under CA storage conditions, are essential for the maintenance of important chemical and physical characteristics of super sweetcorn. The current study aimed to elucidate the temporal changes in textural and taste-related characteristics of seven super sweetcorn cvs. (*viz.* Bob 1, Bob 2, Bob 5, 7210, 6800, Primer Plus and Conqueror) held at 3°C and under controlled atmosphere (CA) (8kPa O<sub>2</sub> and 12 kPa CO<sub>2</sub>) for 24 days.

Maximum Load (N) measured by the Kramer Shear Cell method, indicated that the changes in the texture of the different cvs. followed different patterns during CA storage. Specifically, cv.6800 was *ca.* 1.3-fold firmer than the rest of the cvs during the 24 days of storage. Dry matter as a proportion of fresh weight (DW/FW) was also significantly different between cvs. and tended to decline after 3 days of storage, being 6% lower at the end of the storage period as compared to initial values. On a dry weight basis, sugar content, of which sucrose was the main component (*ca.* sucrose 80.3%, glucose 11.9% and fructose 7.8%), was significantly different between cvs. and 1.2-fold greater at day 0 than at the end of the storage period. In particular, the concentration of total sugar on a dry weight basis was significantly higher in cvs. Bob 1, Bob 5 and Bob 6. This said, when considering the results on a fresh weight basis, the temporal changes in the sugar profile through storage were dependent on both genotype and storage duration.

**Temporal changes in textural and taste-related characteristics of seven UK-grown *sh2* sweetcorn cultivars stored under controlled atmosphere conditions**

10<sup>th</sup> Controlled and Modified Atmosphere research Conference

(April 04-07, 2009, Antalya, Turkey)

# Cranfield Health



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## Textural Characteristics and Spatial Sugar Profile of Super Sweetcorn Stored with or without Husks.

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### Background

The increase in sweetcorn cob consumption has led to more research into several quality parameters during storage. Sweetness and firmness are the major quality attributes in sweetcorn cobs [1], such that maintaining these is critical. In the fresh sweetcorn market, there is an ongoing dispute over whether sweetcorn cobs sold as naked (husks having been fully removed) are closer to consumer requirements as compared to window stripped cobs with a narrow area free of leaves) retail formats. The relationship between sugar content of edible parts (kernels) and non-edible tissues (shank and core) of cobs has also not been investigated and may help explain the biochemical changes and possible relationship between format and handling conditions. Accordingly, the effect of cob format on postharvest temporal changes in texture and sugars content of edible and non-edible tissues of twin-packed UK-grown super sweetcorn (sh2) cobs of cv. 6800 were evaluated.

### Materials and methods

Naked (without husks) and window stripped sweetcorn cobs of the cv. 6800, were stored at 5°C for 10 days. Replicated samples of kernels, core and shank were taken at regular intervals (days 0, 2, 4, 6, 8, 10). Prior to chemical analysis, frozen tissues were freeze-dried. Sugar content (xyl, sucrose, glucose and fructose) were extracted according to Davis et al. (2007) [2] and quantified using HPLC coupled to a Refractive Index Detector. An universal testing machine (Single Column System 5542, Instron, MA) was used for texture-related measurements (maximum compressive load). Least significant differences (LSD) between types of tissue, format, storage times and the interaction of these factors were analysed through analysis of variance (ANOVA).

### Results

• Results suggest that naked cobs (Figure 1) when compared to window stripped cobs (Figure 1), maintained significantly higher mean values of maximum compressive load, when stored at 5°C for 10 days (Figure 2), indicating that naked cobs were firmer than window stripped.

• The total sugar content (glucose + fructose + sucrose) of kernels was ca. 1.21-fold lower than the core and 1.16-fold lower than the shank (Figure 3).

• Sucrose content in kernels was ca. 1.4-fold greater than in the core and ca. 2.2-fold greater than in the shank (Figure 4). Furthermore, glucose (Figure 5) and fructose content (Figure 6) in shanks was significantly higher than in core and kernels.

• No strong correlation was found between sugar content of kernels, core and shank.

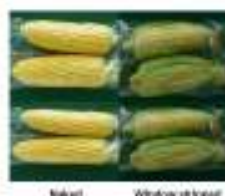


Fig. 1 Naked (left) and window stripped (right) sweetcorn cobs.

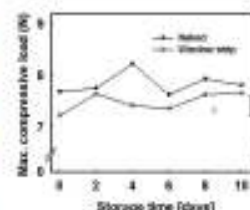


Fig. 2 Means of Max. compressive load (N) of naked and window stripped sweetcorn cobs. (LSD shown: P=0.05).

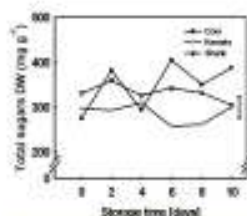


Fig. 3 Temporal changes of total sugar content (mg g<sup>-1</sup> DW) in sweetcorn kernels, core and shank. (LSD shown: P=0.05).

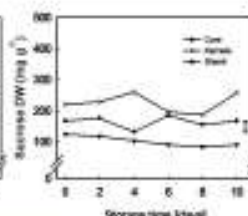


Fig. 4 Temporal changes of sucrose content (mg g<sup>-1</sup> DW) in sweetcorn kernels, core and shank. (LSD shown: P=0.05).

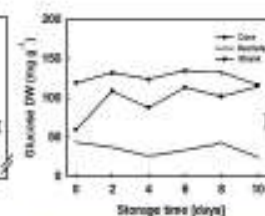


Fig. 5 Temporal changes of glucose content (mg g<sup>-1</sup> DW) in sweetcorn kernels, core and shank. (LSD shown: P=0.05).

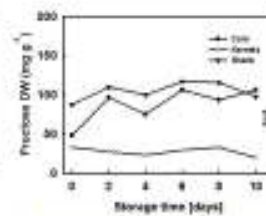


Fig. 6 Temporal changes of fructose content (mg g<sup>-1</sup> DW) in sweetcorn kernels, core and shank. (LSD shown: P=0.05).

### Conclusions

Kernel firmness results was shown to be dependent on the interaction of format (naked cobs vs. window stripped), and storage time. Spatial distribution of sucrose, glucose and fructose, varies strongly between different types of tissue. Sugar profile was dependent on the spatial distribution in kernels, core or shank tissue during storage. However, individual sugar concentrations could not be used to explain a possible relationship between sugar content of edible and non-edible parts of the cobs.

### Acknowledgements

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[1] Terry, W.F. Plant Breeding Reviews, 1987, 14, 255-295.

[2] Davis, P., Terry, L.A., Oliver, G.A., Paul, C.F.J. Journal of Agricultural and Food Chemistry, 2007, 55, 4299-4306.

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## Textural Characteristics and Spatial Sugar Profile of a Super Sweetcorn Stored with or Without Husks.

28<sup>th</sup> International Horticultural Conference, 22-27 August, Lisbon

**Textural characteristics and spatial sugar profile of a super sweetcorn stored with or without husks.**

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**Abstract**

The increase in sweetcorn cob consumption has led to increased research into several quality parameters during storage. Sweetness and firmness are the major quality attributes in sweetcorn cobs such that preserving these is critical. In the fresh sweetcorn market, there is an ongoing dispute over whether sweetcorn cobs sold as naked (husks having been fully removed) are closer to consumer requirements as compared to window strip (cobs with a narrow area free of leaves) retail formats. The relationship between sugar content of edible parts (kernels) and non-edible tissues (shank and core) of the cobs is also not well investigated and may help explain the possible relationship between them, leading to better understand handling conditions. Accordingly, the effect of cob format on postharvest temporal changes in texture and the sugar content of edible and non-edible tissues of UK-grown super sweetcorn (*sh2*) cv. 6800 were evaluated.

Results suggest that naked cobs maintained significantly higher mean values of Maximum Compressive load measured with an Instron Penetrometer, indicating higher firmness than window strip ones when stored at 5°C for 10 days. The total sugar content (glucose+fructose+sucrose) of the kernels was *ca.* 1.21-fold lower than in core and 1.16-fold lower than of shank. Specifically, sucrose content in kernels was *ca.* 1.4-fold greater than in the core and *ca.* 2.2-fold greater than in the shank. Furthermore, glucose and fructose content in shanks was significantly higher than in core and in kernels significantly lower than in core. This said temporal changes in textural profile of the cv. examined were strongly dependent on the format of the cobs and sugar profile on the interaction of tissue (kernels, core or shank) and storage duration.

**Textural Characteristics and Spatial Sugar Profile of a Super sweetcorn Stored with or Without Husks.**

28<sup>th</sup> International Horticultural Conference, 22-27 August, Lisbon

